BOTANY

NEBRASKA
LIBRARY
MAY 28 1926

Volume LXXXI Number 3

The BOTANICAL GAZETTE

FOUNDED BY JOHN M. COULTER

MAY 1926

Revision of the Genus Isostigma Less. Contributions from the Hull Botanical Laboratory 346 - - - - - Earl Edward Sherff 241

Penetration Phenomena and Facultative Parasitism in Alternaria, Diplodia, and Other Fungi - - - - - Paul A. Young 258

Development of Embryo Sac in Grindelia squarrosa Thomas D. Howe 280

Effect of Thiourea upon Bud Inhibition and Apical Dominance of Potato - - - - - - - - - - - - - F. E. Denny 297

Chemical Composition of Etiolated and Green Berberis Sprouts and Their Respective Roots E. R. Schulz and Noel F. Thompson 312

Physiological Investigation of Black Heart of Potato Tuber. Contributions from the Hull Botanical Laboratory 347 - Ward B. Davis 323

Briefer Articles
The Strip Method for Serial Celloidin Sections - Adriance S. Foster 339

Current Literature
Book Reviews - - - - - - - - - - - - - - 342
Notes for Students - - - - - - - - - - - - - 344

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, London
THE MARUZEN-KABUSHIKI-KAISHA, Tokyo, Osaka, Kyoto, Fukuoka, Sendai
THE COMMERCIAL PRESS, LIMITED, Shanghai

Volume LXXXI Number 3

THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of members of the botanical staff of the University of Chicago

Issued May 20, 1926

The Botanical Gazette is published in eight issues annually (omitting January and February, and July and August) by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $9.00 per year; the price of single copies is $1.25. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, El Salvador, Argentina, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, Peru, Uruguay, Paraguay, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, and Spain. ¶Postage is charged extra as follows: For Canada and Newfoundland, 30 cents on annual subscriptions (total $9.30); on single copies 4 cents (total $1.29); for all other countries in the Postal Union, 60 cents on annual subscriptions (total $9.60), on single copies 8 cents (total $1.33). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4, England. Yearly subscriptions, including postage, £2 8*s*. each; single copies, including postage, 6*s*. 8*d*. each.

For China: The Commercial Press, Limited, Paoshon Road, Shanghai. Yearly subscriptions, including postage, $9.60; single copies, including postage, $1.33, or their equivalents in Chinese money.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of the pages in the volume is correspondingly increased. Carbon copies of manuscripts will not be accepted.

Illustrations are furnished without cost to author only when suitable originals are supplied. Plates in excess of four are not accepted unless the author is willing to bear the additional cost. A copy of the suggestions made in the April number, 1916, will be sent on application. It is advisable to confer with the editors as to illustrations required in any article to be offered.

Separates, if desired, must be ordered in advance of publication. A table showing approximate costs of separates is printed on an order blank which accompanies the proof; a copy will be sent on request.

Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

PRINTED IN THE U.S.A

The PRESS IMPRINT

For April 1926 and May 1926

The Municipal Budget

Municipal expenditures have been mounting steadily in recent years, and the problem of city administration has become centered upon the need for reducing expenses. City taxes have gone up so much that the attention of the taxpayer is being turned rather forcibly toward any means by which expenditures may be controlled. The device which promises most for the future is the municipal budget. Yet there has been almost no literature on the subject. R. Emmett Taylor, in *Municipal Budget-Making*, investigates and analyzes budgetary practices in the American city, performing a service for the administrator in city government, the student of government, and the taxpayer who is interested in the reduction of taxes.

This book is a complete discussion of the municipal budget, based upon first-hand investigation of budget-making practices in leading American cities, and upon data secured from questionnaires sent out by the Bureau of Municipal Research, New York City. To study actual procedures, Mr. Taylor visited New York, Boston, Syracuse, Cleveland, Detroit, Columbus, Dayton, Cincinnati, Milwaukee, Minneapolis, St. Paul, Portland, San Francisco, Los Angeles, and Denver.

It is important to note that all the recent changes in municipal budget-making in the United States have been incorporated in this book, and that it is entirely up to date. Since it presents a large amount of data upon a subject which has lacked extensive treatment, it is an exceptionally valuable text or reference for students of municipal government.

From his investigation, the author has formulated conclusions respecting the best procedure to follow in making a municipal budget, dependent, of course, upon the size of the city, the form of government, and local restrictions. His ideas on the subject are suggestive aids in the actual planning and preparation of a city budget.

This comprehensive analysis will give impetus to that development of the municipal budget which is so necessary if expenditures and taxes are to be reduced. *MUNICIPAL BUDGET-MAKING.* By R. Emmett Taylor. *$2.50, postpaid $2.60.*

* * *

The Literature of Spain

A picture of Spanish literature as a whole from its beginning in epic and drama to the present generation of writers is presented in George T. Northup's *An Introduction to Spanish Literature*. A work has been greatly needed to introduce the novice to this national literature, in the way that this has been done for French letters. Mr. Northup's volume has received wide commendation for its scholarship and its interest.

Bulletin of Spanish Studies says, "It is superior to any Spanish history of literature in English, not merely for the place which it gives to movements and tendencies, but in the broadness of the spirit in which it is conceived and written. . . . . It is put together carefully, it criticizes sanely, interprets liberally, and teaches understandingly."

Hispania's comment is "It is no small feat to produce a readable history of Spanish literature which will enlighten and interest the novice, and the present volume stands almost alone in its field."

"An admirable presentation that will be welcomed alike by teachers, students, and the general reader" says *The Modern Language Journal*.

The Bookman comments: "His book is a model of compassion . . . . he has managed to make the story of Spanish literature dramatic and interesting." *AN INTRODUCTION TO SPANISH LITERATURE*. By GEORGE T. NORTHUP. *$3.00, postpaid $3.15.*

* * *

Science Teaching

Modern scientific study of science teaching has had some valuable results, and these Elliot R. Downing summarizes in his new book *Teaching Science in the Schools*. Here is shown how the growing body of facts about the pedagogy of science, which have been ascertained by the experimental method, may be applied practically to the problems of the science teacher.

That science should receive more and more attention in our secondary schools is Mr. Downing's belief. In this book, he points out that science has been introduced into the curriculum comparatively recently, that the percentage of students enrolled in science in the secondary schools is greater than in any other subject, and that the increase in such enrolment in the past three decades has also been greater than in any other subject.

Mr. Downing's discussion includes the history of science teaching, the social and economic background of science teaching, present conditions and aims of science teaching in our elementary and secondary schools, tests in science, and science teaching in some European schools.

From this point the author takes up the principles of selection and organization of science materials, and actual methods of instruction—by book, lecture-demonstration, and laboratory. There is specific information on notebooks and drawings, field trips, and testing results, and suggestions are made for a complete science library in the school. Helpful diagrams and graphs show analysis of zoölogy texts for secondary schools, and enrolment in various high-school science subjects in the United States. *TEACHING SCIENCE IN THE SCHOOLS*. By ELLIOT R. DOWNING. *$2.00, postpaid $2.10.*

* * *

New Light on Old Diseases

The science of paleopathology has revealed the fact that diseases have been man's attendants from remote antiquity. What the evidence supplied by paleopathology shows about the origin and evolution of disease has been summarized by Roy L. Moodie in

The Antiquity of Disease. It constructs a remarkable picture of prehistoric pathological conditions.

Germs, Mr. Moodie finds, are among the oldest inhabitants of the earth, although it is only since the Coal Period that they have been disease-producing. It is probable that they were brought to the earth by meteorites from distant planets.

Early surgical operations began in conjunction with the religious practices of sha-

manism of which the sole aim was to liberate evil spirits. From this arose our modern conceptions which have made surgery such an important factor in modern life. In his investigation of the pathology of the ancient Egyptians, Peruvians, and other primitive peoples, Mr. Moodie finds evidences of such a wide variety of maladies that it becomes apparent that modern man need not envy primitive man as far as health is concerned.

This book is sufficiently non-technical to be read by the layman, and it is a valuable work for those interested in medical history, evolution, zoölogy, and geology. *THE ANTIQUITY OF DISEASE.* By Roy L. Moodie. *$1.50, postpaid $1.60.*

* * *

What Is Religion?

There is no one statement that will define religion, but attempts at definition have a suggestive value. In the revised edition of *The New Orthodoxy*, Edward Scribner Ames has formulated an interesting group of such definitions. From one point of view Mr. Ames sees religion as "A quest for life in an abyss of death," and from another "it is the mirth of kindred spirits round a glowing fire with the shadows playing over a vacant chair."

Explaining his aim in *The New Orthodoxy* Mr. Ames says, "A lady to whom this book was recommended remarked upon reading its title, 'No, thank you, I do not care to read anything that is orthodox.' Her conception was doubtless much the same as that of a 'liberal' who recently wrote: 'All religions tend toward orthodoxy, that is toward something vital and flowing to a static set of doctrines and a cramped attitude of mind.' This he regards as apostasy from 'the principle of free inquiry into all questions bearing upon the religious life.'

"It is the purpose of this book to insist that there has come into the world a new orthodoxy which is precisely the maintenance of this principle of free inquiry. Orthodoxy means right thinking. . . . . It is true that in the past, religious thought, and also all other kinds of thought, sought too much for static, unchangeable truths. But this is no longer considered right thinking. Science in every field has shown the necessity and the advantage of the open mind. . . . . Consequently the tables are completely turned."

Mr. Ames discusses the attitudes and dramatis personae of the new orthodoxy, the growing Bible, the problems of religious sentiments, sacred literature, religious ideals, and ceremonials of worship. *THE NEW ORTHODOXY.* By Edward Scribner Ames. *$1.50, postpaid $1.60.*

* * *

Introductory Sociology

Park and Burgess' widely-used text, *An Introduction to the Science of Sociology*, in its recently revised edition offers a collection of readings selected to define and illustrate the concepts and principles of sociology. An excellent text for an introductory course in sociology, it combines in a single volume the textbook and the necessary reading materials.

Its method of placing concrete material in the students' hands has made possible a clearer presentation of the principles of sociology than has been attained with previous methods. The material is skilfully woven together with introductory and interpretative passages. Each chapter is carefully planned to show the student how to get facts. More than a collection of readings, the book is a systematic treatise, which frees the student from dependence upon library equipment.

Taken from such writers as Thorndike, Sumner, Binet, John Dewey, Georg Simmel, Comte, Spencer, Hobhouse, and Galton, the material represents the investigation and thought of leading figures in sociology and the social sciences. The selections cover the entire field of sociology. In this edition is contained the most extensive and up-to-date sociological bibliography available. New selections have been added to the readings, and revisions in the text have brought it into accord with recent developments in the field.

The discussion shows at once the *structure* of society and the *processes* by which this social structure and the human motives which it embodies grow up, are formed,

The Practice of Teaching in the Secondary School

By

HENRY C. MORRISON

The title of this book indicates its scope only when one understands Professor Morrison's conception of the secondary school. He defines it to be that region in the process of education within which the pupil is capable of study but incapable of systematic intellectual growth without the constant aid of the teacher—that is, the period from about the beginning of the fourth grade to the end of the junior college.

In his service as high-school teacher and principal, city superintendent, state superintendent, and finally for the last six years as a professor in the University of Chicago, with the resources of the Laboratory Schools at his disposal, Professor Morrison has been approaching the present formulation of his critical and constructive contribution to secondary education. The result is the most exhaustive analysis of teaching in the field of general education which has appeared in English.

Criticizing the present methods of education in so far as they are based upon the "lesson-teaching" theory, Professor Morrison outlines a new technique designed for direct teaching of the true learning products. Here is a book for administrators by an administrator, and for teachers by one who has spent twenty-five years in the experimental study of teaching.

$4.00, postpaid $4.15

THE UNIVERSITY OF CHICAGO PRESS

CHICAGO . . . ILLINOIS

and re-formed under the force of changing conditions. Bringing together the observations and reflections of men who have seen life from different angles, this work will interest not only the student who wants a deeper insight into the nature of society but also the layman who seeks a wider knowledge of human affairs.

"This volume will prove a godsend to teachers of sociology. . . . . This ripe and scholarly work is the last word in perfection in source books for the classroom, and no teacher of sociology can afford to be without it."—PROFESSOR E. A. Ross in *The American Journal of Sociology*.

Emory S. Bogardus, editor of the *Journal of Applied Sociology*, says, "The authors have put sociologists under great obligation by collecting source materials from a wide range of investigation. These materials have been excellently arranged and scientifically interpreted. The book offers a fundamental group of sociological concepts as working tools for the student and investigator alike." *AN INTRODUCTION TO THE SCIENCE OF SOCIOLOGY*. (Revised Edition.) By ROBERT E. PARK and ERNEST W. BURGESS. *1,000 pages, $4.50, postpaid $4.70.*

* * *

Thomas Hardy

In making a specialized study of Thomas Hardy's novels exclusively with reference to their technique, Joseph Warren Beach leads the student of literature along paths of technical study long employed with the short story and the drama, but neglected in the case of the novel. *The Technique of Thomas Hardy* is not only a guide to the study of the last of the great Victorian masters, but also throws light upon the general subject of the technique of the novel.

Mr. Beach takes up in succession fourteen Hardy novels, showing the prevailing method of each, and the progress of the novelist's power from mere artifice to genuine art. The development of Hardy's art is traced from the Collins-like ingenuity of *Desperate Remedies*, through the introduction of irony in *A Pair of Blue Eyes*, to a full realization of the value of setting in *Far from the Madding Crowd*.

Mr. Beach raises pertinent questions of Hardy's real attitude toward his art, of the

ideals he held for a novel, and the extent to which he was willing to sacrifice these ideals for the sake of popularity. *The Nation* says of the book: "Mr. Beach's book is intelligent and searching. It fetches up from the depths of your memory all that is lodged there relating to Thomas Hardy." *THE TECHNIQUE OF THOMAS HARDY.* By JOSEPH WARREN BEACH. *$2.50, postpaid $2.60.*

* * *

Francesca da Rimini

Pellico's friend, Ugo Foscolo, advised him to throw the manuscript of *Francesca da Rimini* in the fire, and not terrify the living by recalling Dante's spirits from hell. But Pellico resisted the suggestion, and his became one of the best-known versions of the Francesca story.

Kenneth McKenzie has edited this version, and has included in the same texts extensive selections from Pellico's *Le mie prigioni*, which is perhaps the most famous bit of autobiographical narrative written in nineteenth-century Italy. Its style, patriotic spirit, and fine personal quality all commend it to the student of Italian. The slightly old-fashioned coloring of Pellico's language makes these works a particularly good introduction to the reading of classic Italian literature. *PELLICO'S LE MIE PRIGIONI AND FRANCESCA DA RIMINI.* Edited with Introduction, notes, and vocabulary by KENNETH MCKENZIE. *Illustrated, $1.25, postpaid $1.35.*

* * *

Some Comments

The following sentences about Morrison's *The Practice of Teaching in the Secondary School* are taken from a review by Dr. Winfred E. Garrison in the April 1 *Christian Century*.

"I venture the opinion that it should also have a very wide reading among parents, ministers, students of religious education, and others who have reason to need to understand the educational process even though they may not spend their days behind a teacher's desk. For while the title gives promise of a practical treatment of specific problems of high-school teaching—a promise which is richly fulfilled—the implications touching the meaning of the

The University of Chicago School Science Series

By ELLIOT R. DOWNING

OUR LIVING WORLD. A Source Book of Biological Nature-Study. Cloth, $2.50, postpaid $2.60; flexible, $3.50, postpaid $3.60.

A FIELD AND LABORATORY GUIDE IN BIOLOGICAL NATURE-STUDY. $1.50, postpaid $1.60.

OUR PHYSICAL WORLD. A Source Book of Physical Nature-Study. Cloth, $2.50, postpaid $2.60; flexible, $3.50, postpaid $3.60.

A FIELD AND LABORATORY GUIDE IN PHYSICAL NATURE-STUDY. $1.50, postpaid $1.60.

A NATURALIST IN THE GREAT LAKES REGION. Cloth, $2.50, postpaid $2.60; flexible, $3.50, postpaid $3.60.

TEACHING SCIENCE IN THE SCHOOLS. $2.00, postpaid $2.10.

By W. L. EIKENBERRY

THE TEACHING OF GENERAL SCIENCE. $2.00, postpaid $2.10.

THE UNIVERSITY OF CHICAGO PRESS

CHICAGO · · ILLINOIS

Methods in Plant Histology

Fourth Edition

By

CHARLES JOSEPH CHAMBERLAIN

So extensive have been Professor Chamberlain's revisions that he has given us practically a new book. Rewritten throughout, it brings up to date the methods for preparing all kinds of plant structure for microscopic study.

Killing and fixing, sectioning, staining, the paraffin, Venetian turpentine, celloidin, and glycerine methods are treated, and chapters on making photomicrographs and lantern slides have been added. Even in the universally used paraffin method there has been improvement in fixing, infiltration, and imbedding, and American stains have been successfully used. The Venetian turpentine method has been brought to a high degree of efficiency, and now occasions little difficulty even for a beginner. The cellulose acetate method, which is entirely new, has also been introduced.

Methods in Plant Histology may be used in classes under an instructor but the directions are explicit, and now, more than ever, it will help the student working alone learn to make first-class preparations.

$3.25, postpaid $3.37

THE UNIVERSITY OF CHICAGO PRESS

CHICAGO · ILLINOIS

whole educational process are as important for intelligent people generally as the technical suggestions are to members of the profession.

". . . . The organization of the material of a given course into 'units' with reference to these objectives is a technical matter in which perhaps only teachers will be interested, but the recognition of the objectives is a matter of importance to all.

". . . . I have no fear that teachers will not have it brought to their attention, but I am solicitous that it shall not be overlooked by the many others who ought to read it but to whom the title will suggest something too exclusively technical. . . . ."

*

"I am certain that teachers of long experience who may, at times, feel that they are not accomplishing all that they would wish, as well as beginners in the profession, will find this book full of stimulating ideas and suggestions. Three years of experimentation with these principles in the teaching of American history have convinced me that Dr. Morrison has developed a theory and practice of education that is not only scientifically sound in theory but which really 'educates' in practice."—D. C. BAILEY, Lyons Township High School, LaGrange, Illinois.

"In *The Practice of Teaching* Professor Morrison clears away many confusing and contradictory problems of education when he enforces and illustrates the principle that all true teaching must enable the pupil to make thorough, effective, and permanent adaptations to the fundamental situations which are met in successful living. For three years I have used his principles with increasing satisfaction in my teaching of general science."—CLIFFORD R. MADDOX, Whiting Junior High School, Whiting, Indiana.

"If there are those who doubt the feasibility of a general technique of teaching for secondary schools let them read Morrison. In fact the mastery technique has been successfully used on even the university level in my own classes."—F. DEAN MCCLUSKY, Director of the Division of Educational Reference, Purdue University, Lafayette, Indiana. *THE PRACTICE OF TEACHING IN THE SECONDARY SCHOOL.* By HENRY C. MORRISON. *661 pages, $4.00, postpaid $4.15.*

A Check-List for This Month

The University of Chicago Press
5750 Ellis Avenue, Chicago, Illinois

GENTLEMEN:

Please send me the following books ☐ for examination, ☐ for which I inclose $_____.

☐ *Municipal Budget-Making.* By R. Emmett Taylor. (*$2.60 postpaid.*)

☐ *An Introduction to Spanish Literature.* By George T. Northup. (*$3.15 postpaid.*)

☐ *Teaching Science in the Schools.* By Elliot R. Downing. (*$2.10 postpaid.*)

☐ *The Antiquity of Disease.* By Roy L. Moodie. (*$1.60 postpaid.*)

☐ *The New Orthodoxy.* By Edward Scribner Ames. (*$1.60 postpaid.*)

☐ *An Introduction to the Science of Sociology.* (Revised Edition.) By Robert E. Park and E. W. Burgess. (*$4.70 postpaid.*)

☐ *The Technique of Thomas Hardy.* By Joseph Warren Beach. (*$2.60 postpaid.*)

☐ *Pellico's Le mie prigioni and Francesca da Rimini.* Edited by Kenneth McKenzie. (*$1.35 postpaid.*)

☐ *The Practice of Teaching in the Secondary School.* By Henry C. Morrison. (*$4.15 postpaid.*)

☐ _____

☐ _____

☐ _____

Name _____

Address _____

_____

7

REVISED EDITION

Evolution, Genetics, and Eugenics

By

HORATIO HACKETT NEWMAN

Attendance at the Dayton trial as a witness for the defence has enabled Professor Newman to describe and interpret it in this new revised edition of his text. He brings the knowledge of the evolutionary biologist to bear upon the present anti-evolution campaign in the United States, and studies it historically, giving the facts about evolution needed for an intelligent attitude toward the controversies.

The discussion of mutation, linkage and crossing-over, inheritance of acquired characteristics, eugenics, and other topics has been brought completely up to date. Here in a single volume is a comprehensive treatment of all the important phases of evolutionary biology that takes account of recent developments.

Original material by Professor Newman weaves together a well-balanced selection of excerpts from such writers as Darwin, Weismann, Romanes, and Castle. This is an excellent text for survey courses in evolutionary biology and a clear treatment for the general reader.

$3.50, postpaid $3.70

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO . . . ILLINOIS

The University of Chicago Science Series

The following volumes are bound uniformly
in maroon cloth, small 12mo.

The Evolution of Sex in Plants. By JOHN MERLE COULTER. x+140 pages. $1.25, postpaid $1.35.

Individuality in Organisms. By CHARLES MANNING CHILD. x+212 pages. $1.50, postpaid $1.65.

The Origin of the Earth. By THOMAS C. CHAMBERLIN. xii+272 pages. $2.00, postpaid $2.10.

Finite Collineation Groups. By HANS F. BLICHFELDT. xii+194 pages. $1.50, postpaid $1.60.

The Biology of Twins. By HORATIO HACKETT NEWMAN. x+186 pages. $1.50, postpaid $1.60.

The Physiology of Twinning. By HORATIO HACKETT NEWMAN. x+232 pages. $1.75, postpaid $1.85.

Food Poisoning. By EDWIN OAKES JORDAN. viii+116 pages. $1.25, postpaid $1.35.

The Electron: Its Isolation and Measurement, and the Determination of Some of Its Properties. By ROBERT ANDREWS MILLIKAN. xiv+294 pages. $2.00, postpaid $2.10.

The Living Cycads. By CHARLES JOSEPH CHAMBERLAIN. xiv+172 pages. $1.50, postpaid $1.60.

Problems of Fertilization. By FRANK R. LILLIE. xii+278 pages. $1.75, postpaid $1.85.

The Origin and Development of the Nervous System from a Physiological Viewpoint. By CHARLES M. CHILD. xviii+296 pages. $1.75 postpaid $1.85.

The Story of the Maize Plant. By PAUL WEATHERWAX. xvi+248 pages. $1.75, postpaid $1.85.

The Antiquity of Disease. By ROY L. MOODIE. xiv+150 pages. $1.50, postpaid $1.60.

Algebras and Their Arithmetics. By LEONARD E. DICKSON. xii+242 pages. $2.25, postpaid $2.35.

Protoplasmic Action and Nervous Action. By RALPH S. LILLIE. xiv+417 pages. $3.00, postpaid $3.10.

THE UNIVERSITY *of* CHICAGO PRESS
CHICAGO · ILLINOIS

VOLUME LXXXI NUMBER 3

THE

BOTANICAL GAZETTE

May 1926

REVISION OF THE GENUS ISOSTIGMA LESS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 346

EARL EDWARD SHERFF

(WITH PLATES XXIII, XXIV)

During the past fifteen years, in the course of a monographic study of the genus *Bidens*, my investigations frequently have reached into the genus *Isostigma*. While a small genus, containing as interpreted by me only eleven known species, it displays considerable range in growth habit and even technical characters of flowers and fruits. Indeed, the only reliable distinction between *Isostigma* and *Bidens* appears to rest upon the nature of the style branches. In *Isostigma* these are elongated and of uniform thickness; in *Bidens* they are usually, although not always (for example, *B. cosmoides* [Gray] Sherff) short, and usually have a swelling at or toward the distal end.

The literature for *Isostigma*[1] appears to begin with *Tragoceras peucedanifolium* Spreng., described in 1826 (Linn. Syst. Veg. Edit. XVI. **3**:576). This was based upon a plant by SELLO from the State of Rio Grande do Sul, Brazil. In 1831, LESSING (Linnaea **6**: 513) founded the genus *Isostigma*. To this he transferred SPRENGEL'S earlier species and added three more species, *I. simplicifolium*, *I. speciosum*, and *I. crithmifolium*. All of these were based likewise upon SELLO'S plants. GARDNER, in 1848 (Hook. Lond. Jour. Bot. **7**:408) described a fifth species of true *Isostigma*, although he wrong-

[1] Except as to *Bidens megapotamica* Spreng., discussed under *Isostigma megapotamicum* (Spreng.) Sherff.

ly called it *Glossogyne brasiliensis*. In 1884, BAKER (Martius Fl. Brasil. **6**[III]:238–242, *pl. 71. fig. 1*) presented a treatment of the genus which included two additional names, *I. stellatum* (referable to *I. brasiliense*) and *I. dissitifolium;* also a variety previously named by SCHULTZ BIPONTINUS (*I. speciosum* var. *Riedelii*), and which is to be regarded as a valid species, *I. Riedelii*. In his treatment of *Bidens* (*l.c.*) he erroneously included two more species which must be referred to *Isostigma*, *I. scorzoneraefolium* and *I. acaule*. Since BAKER'S time, KUNTZE (Rev. Gen. **3**[II]:160. 1898) has described *I. Hoffmannii*, and HERZOG (Fedde Rep. Sp. Nov. **7**:358. 1909) has published a description of *I. Herzogii* Hassl. In 1916, HASSLER (Fedde *l.c.* **14**:277) published certain notes upon *Isostigma*, describing varieties that will receive mention under their respective species.

Since no monograph heretofore has been published for this genus, it has seemed to me advisable to bring together a treatment of all the species known so far. In former days, before contemplating the present revision, I examined many of the specimens in the larger European and American herbaria. As a rule, no record was saved of such specimens, and so they cannot be cited in the present revision. Recently, however, I have been lent considerable material expressly for this work. For the great kindness and courtesy shown me in this connection, I wish to express my sincere gratitude to the following botanists: Dr. JOHN BRIQUET, Director of the Botanical Garden of Geneva; Dr. H. DIELS, Director of the Berlin Botanical Garden; Dr. A. W. HILL, Director, and Mr. A. D. COTTON, Keeper of the Herbarium, at the Royal Botanical Garden of Kew; Professor BORIS L. ISSATSCHENKO, Director of the Botanical Garden of Leningrad (Hort. Petropol.); Professors H. LE COMPTE and PAUL DANGUY, of the Museum of Natural History, Paris; Mr. W. R. MAXON, Associate Curator of the United States National Herbarium; Dr. B. L. ROBINSON, Curator of Gray Herbarium; Dr. E. DE WILDEMAN, Director of the Botanical Garden at Brussels.

Photographs of most of the plants cited are in my private collection, and duplicates are in the Herbarium of the Field Museum of Natural History.

ISOSTIGMA Less., Linnaea **6**:513. 1831.—Herbae perennes vel basi suffrutescentes, saepe e rhizomate lignoso crasso brevi vel elongato ramosoque, glaberrimae, caulibus simplicimis vel moderate ramosis. Folia infra plerumque numerosa et ad basim caulis plus minusve rosulata, supra pauciora et saepe reducta et etiam squamis non dissimilia, omnia nunc integra et linearia lanceolatave, nunc dissecta segmentis linearibus vel filiformibus, interdum valde elongatis. Capitula saepe magna speciosaque, plerumque heterogama radiataque, floribus ligulatis feminea, tubulosis hermaphrodita, rarissime radio deficiente homogama. Involucrum campanulatum, plerumque 2- rarius 3-∞-seriale, bracteis intimis 1-seriatis membranaceis subaequalibus, exterioribus (plerumque brevioribus) angustis, omnibus basi breviter connatis. Receptaculum planum, paleis scariosis planis vel concaviusculis flores tubulosos subtendentibus onustum. Corollae radii ligulatae, lamina patentes, apice 2-3-dentatae, nunc extra atro-purpurascentes et intus plus minusve albido-argenteae, nunc omnino luteae vel luteo-rubrae, saepe parvae; florum hermaphroditorum regulares, tubulosae, limbo ampliato, cylindraceo, 4- vel 5-fido. Antherae basi obtusae, subintegrae. Styli florum hermaphroditorum rami angusti, in appendices longas subulatas hirtas desinentes. Achaenia obcompresso-tetragona vel plana, linearia vel oblonga, faciebus striata, glabra vel sursum setulosa, margine nunc exalata nunc tenuia vel saepius anguste alata, apice truncata, aristis 2 rectis divaricatisve, longiusculis vel brevissimis, laevibus vel sursum scabridis coronata.[2]

CLAVIS SPECIERUM

Folia indivisa (nisi ad apicem breviter furcata)
  Folia oblanceolata, ad basim caulis (pedunculi) dense rosulata, apice 3–5-dentata ..... 2. *I. acaule*
  Folia angustiora
    Folia subulata, ad basim pedunculi dense rosulata ..... 1. *I. brasiliense*
    Folia latiora, partim caulina
      Flores flavi vel lutei ..... 3. *I. scorzoneraefolium*
      Flores atro-purpurei
        Capitula solitaria

[2] Generic description based, with various modifications, upon that given by BENTHAM and HOOKER, Gen. Pl. **2**:389. 1876.

Caulis versus apicem plerumque plurisquamatus; ligulis longis perspicuisque.....10. *I. Riedelii*
Caulis versus apicem fere vel omnino esquamatus; ligulis disco subaequantibus.....5. *I. simplicifolium*
Capitula in corymbis 3–4-floris.....4. *I. Herzogii*
Folia divisa
Capitula discoidea, minuta, demum 7–9 mm. alta et 6.5–10 mm. lata 1. *I. brasiliense*
Capitula radiata, pansa ad anthesin 1–2 cm. alta et 2.5–3.5 cm. lata 6. *I. Hoffmannii*
Capitula plerumque radiata, majora
Pedunculus subrobustus, altus, ad apicem 4–5 mm. crassus et perspicue squamatus.....11. *I. speciosum*
Pedunculus gracilior, ad apicem plerumque tantum 1–3 vel nullis squamis praeditus
Folia caulina plura segregata.....8. *I. dissitifolium*
Folia ad basim caulis dense rosulata
Segmenta subulata 0.2–0.7 mm. lata.....9. *I. megapotamicum*
Segmenta linearia 0.5–1.3 mm. lata.....7. *I. crithmifolium*

1. ISOSTIGMA BRASILIENSE (Gardn.) Benth. and Hook. *ex* Ind. Kew. **2**:1240. 1893; *Glossogyne brasiliensis* Gardn., Hook. Lond. Jour. Bot. **7**:408. 1848; Walp. Ann. **2**:869. 1851–52; *Isostigma stellatum* Baker, Martius Fl. Brasil. **6**^{III}:239. 1884; *I. microcephalum* Baker, *l.c.*—Pl. XXIII, figs. *n*, *o*, *p*, *q*, *r*, *s*, *t*.—Herba basi fruticulosa, 1–3 dm. alta, caulibus demissis, perspicue ramosis, ramis confertis, dense caespitosis. Folia ad basim pedunculi dense rosulata, nunc simplicia, strictiuscula, apice mucronata, 2.5–7 cm. longa et circ. 0.2 mm. lata; nunc ternatim biternatimve secta, plerumque 1.5–3 cm. longa, segmentis acerosis, apice mucronatis, (siccis) circ. 0.2 mm. latis. Capitula parva, discoidea, longe pedunculata, demum cum fructibus (aristis inclusis) 5–9 mm. altis et 4–10 mm. latis, pedunculis monocephalicis, strictis, erectis, usque ad 2.5 dm. longis, ad medium unisquamosis vel etiam supra basim foliis unijugis simplicibus vel compositis praeditis. Involucrum campanulatum bracteis omnibus adpressis, rigidulis, brunneis, exterioribus circ. 3 vel 4, parvis, lineari-oblongis, 1–1.5 mm. longis; interioribus late lanceolatis, margine magis perspicue diaphanis, 2–4 mm. longis. Achaenia linearia, plana vel subtetragona, fusco-atra, supra faciebus marginibusque erecto-hispida, corpore 3–5 mm. longa et 0.3–0.9 mm. lata,

apice biaristata aristis adscendentibus, tenuibus, erecto-scabridis, 1–2 mm. longis.

Type specimen: *George Gardner* 4253, near Senhora d' Abbadia, State of Goyaz, Brazil, in 1841 (Herb. Kew).

Distribution: States of Goyaz and Sao Paulo, Brazil.

Specimens examined: *G. Gardner* 4253 (Herb. Kew, type; Herb. Gray); *A. Glaziou* 21557, Cassú, Goyaz, August 5, 1894 (Herb. Berl.); *A. T. Regnell* ser. III. 738 (per errorem *Baker* 783 dixit), Sao Paulo, February, 1847 (Herb. Berl.); *anon.* (verisimiliter *Riedel*) 98, Paraná River, Brazil (Herb. Deless.) *L. Riedel* 410, in saxosis fissuris rupium ripae fluminis Paraná, prope Urubupunga, Brazil (Herb. Gray; Herb. Kew).

BAKER distinguished his *Isostigma stellatum* from *I. brasiliense* by means of the leaves, referring to the former those with simple leaves and to the latter those with tripartite leaves. Incidentally, he described the peduncles for the former as 5–10 cm. ("2–4 poll.") long, and for the latter as 15–22.5 cm. ("6–9 poll.") long. These distinctions are seen not to hold among further specimens examined by me. Thus, while *Riedel* 410 (*ex* Herb. Petrop.) at Kew has the leaves all simple, the anonymous 98 (*ex* Herb. Petrop. in Herb. DC.) at Geneva, a specimen which probably also was collected by RIEDEL, has the leaves slightly longer, namely, 4–7 cm. long, and the many leaves of both simple and tripartite types. Of the more recent Glaziou specimens (five on one sheet), two have mainly tripartite leaves and three have also great numbers of simple leaves, in some cases the leaf clusters being composed exclusively of a score or more of simple leaves. Furthermore, the Glaziou plants, as also the REGNELL plant at Berlin (of which BAKER cited a duplicate for *I. brasiliense*), have short peduncles as in BAKER's material of *S. stellatum*. Thus the length of the peduncles as well as the nature of the leaves is seen to be a fickle character. An examination of other characters fails to show any grounds for treating the several forms as other than members of one species.

2. ISOSTIGMA ACAULE (Baker) Chod., Bull. Herb. Boiss. ser. II. 1:417. 1901; *Bidens acaulis* Baker, Martius Fl. Brasil. 6^{III}:247. 1884; *Isostigma Vailiana* Britt. in MORONG and BRITTON Enum. 149. 1892.—Pl. XXIII, figs. *a*, *b*.—Herba 1.5–2.5 dm. alta, caulibus basi decumbentibus. Folia plura, rosulata, subpetiolata, rigidula, cuneato-oblanceolata, apice plerumque 3 (interdum–7)-dentata, 1.5–7 cm. longa et 4–12 mm. lata. Capitula solitaria caules elongatos terminantia, discoidea, demum circ. 1 cm. alta et 1.5 cm. lata. Involucrum campanulatum, bracteis adpressis, rigidulis, anguste lanceolatis, acutis, viridulis margine pallidis, subaequilongis (5–7 mm.). Flores disci saturate purpureo-rubri, 4-dentati. Achaenia linearia, sub-

tetragona, hispidula, corpore 8–10 mm. longa et circ. 1 mm. crassa, biaristata aristis scabris 2–3 mm. longis.

Type specimen: *B. Balansa* 913, in clay soil, Plain of Arégua, Paraguay, January, 1875 (Herb. Kew).

Distribution: Paraguay.

Specimens examined: *B. Balansa* 913 (type in Herb. Kew; cotype, Herb. Hort. Petrop.); *Dr. Emil Hassler* 1030, in field near Tacuaral, Paraguay, September, 1885–95 (Herb. Hassl. in Herb. Deless.); *idem* 3811, in field at Tacuaral, Paraguay, January, 1899 (Herb. Hassl. in Herb. Deless.); *Thomas Morong* 734, Limpio, central Paraguay, May, 1888–1890 (Herb. Gray; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.).

A species with the general aspect of *Glossogyne tridentata* (Turcz.) B. and H. *ex* Ind. Kew. of the Philippine Islands, but growing considerably larger.

3. **Isostigma scorzoneraefolium** (Baker), comb. nov.; *Bidens scorzoneraefolia* Baker, MARTIUS Fl. Brasil. **6**^{III}:247. 1884; *Isostigma glycinaefolium* (Schz. Bip. *ex* Baker) SHERFF, BOT. GAZ. **76**:78. *pl. 6.* 1923; *Bidens glycinaefolia* Schz. Bip. *ex Baker, l.c.; Isostigma foliosum* Malme, Svensk. Vet. Akad. Handl. **32**^{v}:66. *pl. 6. fig. 17.* 1899.—Pl. XXIII, figs. *g, h.*—Herbacea, 5–7 dm. alta; caule simplici nisi versus apicem, subtereti, inconspicue angulato, supra sparsim folioso vel subnudo, infra dense folioso, e basi lignosa. Folia glaucescentia, eis graminearum valde similia, sessilia, inferiora 8–19 cm. longa et 4–10 mm. lata, conferta, superiora sensim minora et distantia, omnia alterna vel perpauca opposita, indivisa, linearia, elongata, apice acuminata, infra angustata, basi ipsa plerumque dilatata atque amplexicaulia, integra vel parce antico-serrata dentibus angustis acribusque, rigidula et parce membranacea. Capitula pauca (circ. 4–9), laxe corymbosa, ramulos 3–14 cm. longos coronantia, radiata, pansa ad anthesin 2–2.5 cm. lata et fere vel etiam 2.5 cm. alta. Flores ligulati 8–14, flavi luteive, ligula lineares, apice acriter bidentati, 9–13 mm. longi; paleis versus apicem perspicue longo-attenuatis, achaeniorum corpora superantia; disci floribus ad apicem acriter 4-dentatis; disco demum circ. 12 mm. lato et 6–9 mm. alto. Involucrum glabratum vel minutissime pubescenti-scabridum tuberculo-rugulosumve, bracteis plus minusve 3-seriatis, rigidulis, adpressis, lanceolatis, margine saepe erosis et etiam ciliatis, extimis 3–7, acutis, 3–5 mm. longis; interioribus lanceolatis, ad apicem saepe attenuatis, paulo longioribus. Achaenia linearia vel anguste lineari-

cuneata, plana vel obcompresso-tetragona, fusco-atra vel rubronigrescentia, exalata, marginibus ac costis medianis suberecte setosa, alibi glabra, corpore 5–8 mm. longa et circ. 1 mm. lata, biaristata; aristis tenuibus, erectis, calvis vel versus basim arrecte setulosis (vel omnino rigide ciliatis ex Baker), 1–2 mm. longis.

Type specimen: *Patricio Da Silva Manso* 215, at Cuyabá, State of Mattogrosso, Brazil, 1834 (Herb.[3] Brüss.).

Distribution: Vicinity of Cuyabá, State of Mattogrosso, Brazil.

Specimens examined: *Gust. Malme* 1584, in sandy-gravelly, grassy, subhumid, open field near Coxipó (igreja), vicinity of Cuyabá, April 26, 1894 (cotypes of *I. foliosum* Malme; Herb. Berl.; Herb. Brit. Mus.); *Patricio Da Silva Manso* 215, Cuyabá, 1834 (type in Herb. Bruss.); *R. Pilger* 479, scattered upon stony ground at foot of Serra das Pedras in Cuyabá Valley, April 15, 1899 (Herb. Berl., two sheets); *L. Riedel*, in subhumid, grassy places, near Cuyabá, May, 1827 (Herb. Kew; Herb. Par.; Herb. Petrop.).

Baker described his *Bidens scorzoneraefolia* from the upper portion of Manso's plant, collected at Cuyabá. Had Baker seen an entire plant with its interesting basal leaves, or had he at least known that the type of *B. glycinaefolia*[4] was collected at the same station, he doubtless would have been led to compare the two. In any case, Baker's description shows that he was dealing with the same species which he described, further down on the same page, under the name *Bidens glycinaefolia* Schz. Bip.

Malme's *Isostigma foliosum*, unknown to me until recently, is seen from its cotypes and type illustration to be synonymous with this species, and, moreover, to have been collected in *I. scorzoneraefolium's* type locality, Cuyabá.

4. Isostigma Herzogii Hassl., Fedde Rep. Spec. Nov. **7**:358. 1909.—Pl. XXIV, figs. *i, o, r, t, u*.—Herba 5–6 dm. alta, caule simplici ramulis simplicibus apice floriferis paucis aucto, tereti, manifeste et prominenter striato, ad basim circ. 3 mm. crasso. Folia basalia verisimiliter more generis rosulata (in typi specimine unico incompleto folium basale unicum adest); caulina vulgo alterna, rarius plus minusve opposita, distantia internodiis 2–5 cm. longis, basali similia sed minora, omnia lineari- vel oblongo-lanceolata, basi sessilia

[3] Baker did not cite the herbarium possessing the type, but recently, through the aid of Dr. Karl Goebel of Munich, I was able to locate it among the Manso plants sent to Von Martius and deposited now in Brussels. I am indebted to Dr. E. De Wildeman for his having allowed me to make an examination of the specimen.

For interesting information as to the type locality, Cuyabá, the reader is referred to S. L. Moore, Trans. Linn. Soc. 2d Ser., Bot. **4**:265–270 *et sequ.* 1895.

[4] For an extended discussion of the type of *Bidens glycinaefolia*, see Sherff, Bot. Gaz. **76**:78. 1923.

semiamplexicaulia, apice acuta, subcoriacea, concoloria, margine eburneo cincta basale ±6.5 cm. longum et ±6 mm. latum, caulina 2–4 mm. lata. Capitula ad apices caulis et ramulorum in corymbis 3–4-capitulis, odore (fide Herzog) Nigritellae similia, satis longe pedicellata, pedicellae bracteis 1–3 foliis caulinis similibus sed minoribus. Involucrum urceolato-campanulato, bracteis exterioribus anguste triangularibus, crassiusculis, leviter reflexis, margine albicanti subhyalino lacerato-fimbriato cinctis, 3 mm. longis et 0.75 mm. latis; interioribus oblongis vel ovato-oblongis, tenuioribus, margine latiusculo ei bractearum exteriorum simili cinctis, 5–6 mm. longis et 1.2–2 mm. latis. Flores ligulati atro-purpurei, ligula 9–11 mm. longi, stigmatibus longe appendiculatis, aequilongis, ovario oblongo sparse setuloso, 3 mm. longo et 1 mm. lato, aristis subulatis, patulis 1.5–2 mm. longis; paleis linearibus, apice acutis, 8–9 mm. longis et vix 0.2 mm. latis. Achaenia oblonga, versus basim angustata, plana vel obcompressa, flava, medio superiore setulis sparsis hispidula, 12 mm. longa et 1 mm. lata, aristis subulatis 2 mm. longis.

Type specimen: *Dr. Theodor Herzog* 617, abundant in field, alt. about 600 m.. Santiago de Chiquitos, Dept. of Santa Cruz, Bolivia, May, 1907 (Herb. Hassl. in Herb. Deless.).

Distribution: Known only from type locality in southeastern Bolivia.

Specimens examined: *Herzog* 617 (type).

The tiny fragment studied by HASSLER was much too scanty to afford a broad and adequate idea of the species as a whole. It was only about 1.5 dm. long. The species would seem, however, to be like *I. scorzoneraefolium* in habit, but like *I. Hoffmannii* in color of its rays.

5. ISOSTIGMA SIMPLICIFOLIUM Less., Linnaea **6**:513. 1831.—Pl. XXIII, figs. *c*, *d*, *e*, *f*, *v*, *w*.—Herba erecta, 3–6 dm. alta, caulibus paucis e rhizomate crasso lignoso, simplicibus. Folia ad basim pedunculi longi dense rosulata, alterna, simplicia, sublinearia, basi longe angustata, coriacea, nervis parallelis multis striata, apice acuto vel obtusiusculo plus (usque ad 5-) minusve denticulata, nonnumquam obsolete trifida, plus minusve curvata, nitida, 7–13 (vel etiam–25) cm. longa et 1.5–5 mm. lata. Capitula solitaria, ligulata, pedunculis 1–2 (vel etiam usque ad 6)-squamatis, florescentia (ligulis non inclusis) circ. 1.5–2 cm. latis et circ. 1.3 cm. alta. Involucrum campanulatum, biseriale, glabrum, bracteis exterioribus circ. 8–10, linearibus, acutis, circ. 2.5–4.5 mm. longis, interioribus

lanceolatis margine scariosis, apice subobtusis et irregulariter plus minusve dentatis, circ. 10–12 mm. longis. Flores ligulati magis minusve profunde 3-partiti, atro-purpurei, disco subaequantes; disci atro-purpurei. Achaenia linearia, nitida, incurva, striata, circ. 12 mm. longa et 2 mm. lata, anguste alata, alis integerrimis in aristulas binas, tenues, laevissimas, latitudinem ovarii aequantes excurrentibus.

Type specimen: *Sello* 873 (numbered also 1111), Facienda da Porteira, in 1818 (Herb. Berl.).[5]

Distribution: Eastern Brazil.

Specimens examined: *Claussen*, in fields at Cachoeira do Campo, Minas Geraes, Brazil (Herb. Kew); *Sello* 873 and 1111 (3 type sheets, Herb. Berl.).

6. ISOSTIGMA HOFFMANNII O. Kuntze Rev. Gen. 3^{II}:160. 1898. —Pl. XXIV, figs. *h*, *p*, *q*, *s*.—Herba suffrutex, parvus, e rhizomate lignoso, tantum 1–4 dm. alta, caulibus simplicibus, infra plerumque arcuatis, supra nudis vel remotissime 1–3-squamatis. Folia nunc ad basim caulis dense rosulati, nunc per caulem inferiorem opposite alterneve disposita, petiolata petiolis subangustis vel alatis usque ad 2.5 cm. longis et ad basim saepe connatis, petiolo adjecto 1.5–5.5 cm. longa, plus minusve bipinnati- vel biternatisecta, costa mediana segmentisque linearibus, planis, 0.5–3 mm. latis, apice acutis vel etiam acuminatissimis. Capitula solitaria, moderate parva, circ. 50–60-flora, longe pedunculata, radiata, pansa ad anthesin 2.5–3.5 cm. lata et 1–2 cm. alta. Involucrum campanulatum, bracteis irregulariter 2–4-seriatis, adpressis, exterioribus linearibus, viridibus, apice acutis, margine ciliatis, 3–5 mm. longis, intimis lanceolatis, plerumque 4–6 mm. longis. Flores ligulati, parvi, fusco-rubri vel luteo-rubri, ligula lineares, apice bidentati, 6–9 mm. longi; tubulosi apice 4-dentati. Achaenia linearia, obcompressa, atro-fusca, marginibus et nervis et apice erecto-setosa, margine anguste alata, cor-

[5] One of the three sheets in Berlin has the locality, Facienda da Porteira. A second gives merely Brasilia. The third bears two specimens (*a Humboldt ex reliquiis Selloianis anno 1836 datis*). One of these was correctly named *I. simplicifolium* and has a label similar to that for the first (Facienda da Porteira, etc.). The other was unnamed and without a number, but is *I. crithmifolium*, and the locality is S. Antonio do Monte. It is perhaps from this last plant that BAKER obtained the locality S. Antonio do Monte for *I. simplicifolium*, a species to which he may have referred the plant of *I. crithmifolium*. BAKER (Martius Fl. Brasil. 6^{III}:240. 1884) cited also S. Antonio do Monte for *Sello's* 873 and 1111.

pore 6–10 mm. longa et circ. 1 mm. lata, perspicue biaristata aristis tenuibus, divergentibus, omnino laevissimis vel versus basim erecto-barbellatis, 3.5–5 mm. longis.

Type specimen: *Dr. Otto Kuntze*, alt. 400 m., Rio Yapacani, Dept. of Santa Cruz, Bolivia, June, 1892 (Herb. Berl., 2 sheets).

Distribution: Southern Bolivia and northern Argentina.

Specimens examined: *Alcide D' Orbigny* 639, Dept. of Santa Cruz, Bolivia, May, 1826–1834 (Herb. Par.); *Th. Herzog* 234, alt. about 400 m., abundant on the savanna pampas of Santa Cruz de la Sierra, Dept. Santa Cruz, Bolivia, November, 1907 (Herb. Berl.); *Dr. Otto Kuntze*, Rio Yapacani, Dept. Santa Cruz, Bolivia (2 type sheets, Herb. Berl.; Herb. U.S. Nat.); *P. G. Lorentz*, Prov. of Santiago del Estero, Argentina, February, 1874 (Herb. Berl., *sub nom. I. Lorentzii* Hieron.); *H. Alg. Weddell* 3569, Santa Cruz de la Sierra, Dept. Santa Cruz, Bolivia, November, 1845 (Herb. Par.).

KUNTZE (*l.c.*) described the disk florets as 5-dentate at the top, but I have found only 4-dentate florets in his type material. HIERONYMUS (in Herb. Berl.) had remarked upon this same 4-dentate character in the LORENTZ material, and had given LORENTZ's plant the name (apparently heretofore unpublished) *Isostigma Lorentzii*.

7. ISOSTIGMA CRITHMIFOLIUM Less., Linnaea **6**:515. 1831; BAKER, MARTIUS Fl. Brasil. **6**^{III}:241. *pl. 71. fig. 1.* 1884.—Pl. XXIV, figs. *b*, *c*.—Herba erecta, 5–7 dm. alta, caulibus caespitosis, simplicibus, tenuibus, remotissime squamulatis. Folia ad basim caulis (pedunculi) dense rosulata, petiolo adjecto 1.2–2.5 dm. longa, in dimidio superiore 5–7-furcata, foliolis linearibus, inferioribus rursus furcatis, segmentis planis, 0.5–1.3 mm. latis, ultimis segmentis 2–6 cm. longis. Capitula solitaria, moderate magna, radiata, pansa ad anthesin circ. 5 cm. lata et 1.8–2.3 cm. alta, sub basi solum 1–2 squamulis praedita. Involucri campanulati bracteae exteriores 7–10, virides, adpressae, lineares, plerumque e basi usque ad apicem acutum sensim angustatae, 3–6 mm. longae; interiores lanceolatae, margine scariosae, 10–15 mm. longae. Flores ligulati circ. 12–15, extra brunneo-purpurei intus plerumque albido-argentei lineares, apice plerumque 3-dentatae, 1.8–2.5 cm. longi. Achaenia (fide Baker) linearia, 1.6–1.8 cm. longa, aristis minutis patulis lanceolatis.

Type specimen: *Sello*,[6] Uruguay, in 1823 (Herb. Berl.).

[6] Lessing cited no particular number of SELLO's. The Berlin Herbarium contains four sheets of SELLO material from *Brasilia* (as then bounded). Two sheets are labeled "Salto, Febr., 23" and one is labeled "S. José del Uruguay, Feb., 23," whence it is seen that SELLO collected the types in what is now Uruguay.

Distribution: Uruguay and Province of Entre Rios, Argentina.

Specimens examined: *Dr. P. G. Lorentz* 862, in pastures, between Yucari grande and Chico, Province of Entre Rios, Argentina, February 20, 1876 (Herb. Berl.); *Sello*, Uruguay (4 sheets of type material, Herb. Berl.).

8. ISOSTIGMA DISSITIFOLIUM Baker, MARTIUS Fl. Brasil. 6^{III}: 239. 1884; *I. peucedanifolium* var. *dissitifolium* (Baker) Hassler, Fedde Rep. Spec. Nov. **14**:277. 1916.—Pl. XXIV, figs. *a*, *f*, *j*.—Herba erecta, caulibus multis caespitosis parce ramosis, 0.6–1 m. altis. Folia caulina plura segregata, opposita vel alterna, petiolata petiolis tenuibus 1–3 cm. longis, petiolo adjecto 5–10 cm. longa, 5–7-furcata, segmentis lineari-subulatis 0.5–1 mm. latis, inferioribus furcatis. Capitula solitaria, radiata, pansa ad anthesin 4–5 cm. lata et 1–2 cm. alta, longissime pedunculata pedunculis usque ad 4 dm. longis, foliis 1 vel 2 minutis squamosis praeditis. Involucrum campanulatum, bracteis exterioribus circ. 8–10, anguste linearibus, viridulis, adpressis, 3–10 mm. longis; interioribus oblongo-lanceolatis, rigidulis, griseo-violaceis vel brunneo-purpurascentibus, 8–13 mm. longis. Flores ligulati ±10, extra rubro-brunnei intus plerumque albido-argentei, linearia, apice profunde acerrimeque dentati, 1.5–2 cm. longi. Achaenia linearia, obcompressa, faciebus subatra, marginibus subalatis straminea, omnino glabra, corpore 1.1–1.7 mm. longa et 1.5–2 mm. lata, apice biaristata aristis patulis erectisve tantum usque ad 1 mm. longis.

Type specimen: *B. Balansa* 907, on uncultivated hills, Guarapi, district of Yaguaron, Paraguay, June, 1877 (Herb. Kew).

Distribution: Paraguay.

Specimens examined: *B. Balansa* 907 (type in Herb. Kew); *K. Fiebrig* 450, high, rocky places, among grasses, Cordillera de Altos, Paraguay, November, 1902 (*sub nom. Isostigma Fiebrigii* Hieronymus, Herb. Berl., 3 sheets; Herb. Deless., 2 sheets; Herb. Field Mus.; Herb. Gray, 2 sheets; Herb. Hassl. in Herb. Deless.; Herb. U.S. Nat., 2 sheets); *Dr. E. Hassler* 1074, in field near Itacurubi, Paraguay, September, 1885–95 (Herb. Hassl. in Herb. Deless.); *idem* 1509, sandy fields, San Bernardino, Paraguay, October, 1915 (Herb. Hassl. in Herb. Deless.); *idem* 3944, Paraguay (Herb. Hassl. in Herb. Deless.); *idem* 6309, in region of hills, "Cerros de Tobaty," central Cordillera, Paraguay, September, 1900 (Herb. Berl.; Herb. Gray; Herb. Hassl. in Herb. Deless.).

FIEBRIG (*in herb.*) states that the leaves when rubbed smell like celery. HASSLER (*l.c.*) states that this species differs from *I. peucedanifolium* (Spreng.) Less. (=*I. megapotamicum*) only in having the cauline leaves large and unre-

duced, but the matter of leaf reduction and leaf distribution in *Isostigma* seems a fairly important criterion. I have found no transitions connecting the two forms.

9. **Isostigma megapotamicum** (Spreng.), comb. nov.; *Bidens megapotamica* Spreng. Syst. Veg. **3**:454. 1826; *Tragoceras peucedanifolium* Spreng. (*fide* Less.) *l.c.* 576; *Isostigma peucedanifolium* (Spreng.) Less., Linnaea **6**:514. *pl. 6. figs. 1–5.* 1831.[7]—Pl. XXIV, figs. *g, k, l, m, n, v.*—Herba erecta, 5–10 dm. alta, e rhizomate lignoso, caulibus simplicibus, caespitosis, squamis 1–3 minutis, subulatis, obsitis. Folia ad basim caulis tenuis (pedunculi) dense rosulata, 1.2–3.3 dm. longa (petiolo adjecto), in dimidio superiore 5–7-furcata, segmentis adscendentibus et petiolis similibus, inferioribus plerumque furcatis, ultimis tenuissime linearibus, trigonis, supra canaliculatis, 3–12 cm. longis et tantum 0.2–0.7 mm. latis. Capitula solitaria, plerumque radiata, saepe speciosa, pansa ad anthesin 3–5 cm. lata et 1.7–2 cm. alta. Involucri campanulati bracteae glabrae exteriores 8–16, lineares vel flagelliformes, supra attenuatae, apice acutae, nunc tantum 5–7 nunc etiam usque ad 28 mm. longae, interiores lanceolatae usque ad 2 cm. longae. Flores ligulati circ. 15, extra atro-purpurei intus plerumque albido-argentei, ligula lineares, apice profunde 2–3-dentati, 1.8–2.5 cm. longi; paleis rigidulis, brunneo-purpureis, lineari-lanceolatis, 12–15 mm. longis, quam achaeniis nunc brevioribus nunc longioribus. Achaenia linearia, obcompressa, brunnea vel atro-brunnea, nitida, glabra, striata, 12–18 mm. longa et 1.2–2 mm. lata, anguste alata, apice aristulis binis, marginalibus, laevissimis, patenti-divergentibus, 0.5–2 (rarius usque ad 4.5) mm. longis, interdum deciduis, plerumque coronata.

Type specimen: Collected by SELLO, probably in the State of Rio Grande do Sul, Brazil,[8] but perhaps in Uruguay (no herbarium cited by SPRENGEL, but see later discussion).

Distribution: Eastern and southern Brazil; Paraguay; perhaps also in adjacent portion of Argentina and in Uruguay.

Specimens examined: *Alfred Bornmüller* 761, New Wurttemberg, Rio Grande do Sul, Brazil, December 7, 1906 (Herb. Berl.); *Burchell* 5259, tropical

[7] For *I. peucedanifolium* var. *genuinum* Hassl. *cum* f. *discoideo* Hassl. *et* f. *radiato* Hassl., see under *L. speciosum* Less.

[8] For geographic note in this connection see SHERFF, BOT. GAZ. **76**:92 (footnote 11). 1923.

Brazil (Herb. Gray); *P. Dusen* 3228, in field, Paraná, Brazil, December 14, 1903 (Herb. Berl.); *Dr. A. Glaziou* 20381, Minas Geraes, Brazil, 1893–94 (Herb. Berl.; Herb. Kew); *Dr. E. Hassler* 10366, in dry field, Punta Para, Sierra de Amambay, Paraguay, April, 1908 (Herb. Hassl. in Herb. Deless.); *Gust. A. Malme* 1158, dry, open, sandy-gravelly place, Cuyabá, Mattogrosso, Brazil, November 24, 1893 (Herb. Berl.); *G. Niederlein*, between Corrientes, Argentina, and Santa Ana, Paraguay, January 22, 1883 (Herb. Berl.); *Capt. Page* (La Plata Exped.), Paraná, Brazil (Herb. U.S. Nat., a form with aristae up to 1.5 mm. long); *R. Pilger* 493, upon stony ground at foot of the Serra das Pedras, in Cuyabá Valley, Mattogrosso, Brazil, April 15, 1899 (Herb. Berl.); *A. F. Regnell* ser. III. 782, Uberava, Minas Geraes, Brazil, September 29, 1848 (Herb. Berl.; Herb. U.S. Nat.); *E. M. Reineck* and *Joseph Czermak* 273, sunny slopes of the Parthenon Mountains, Porto Alegre, Rio Grande do Sul, Brazil, December, 1898—January, 1899 (Herb. Berl.; Herb. Deless., 2 sheets); *L. Riedel*, Brazil (Herb. Gray, 2 sheets); *idem* 797, Brazil (Herb. Gray; the slender aristae measuring 1–2 mm. long); *Sello* 1723 *pro parte*, 3327 and 5179 (8 sheets of type material, Herb. Berl.; 3327 also in Herb. Gray); *idem*. Brazil (Herb. Gray; Herb. Kew, 2 sheets); *Tweedie* 165, Rio Jacuhy, Rio Grande do Sul, Brazil, in 1837 (Herb. Kew).

With original, authentically named specimens of *Bidens megapotamica* Spreng., LESSING evidently was not familiar. In his *Synopsis Compositarum*, published (1832) six years after SPRENGEL'S description, he completely omits this name. DE CANDOLLE (Prodr. **5**:604 1836) had seen no authentic material to represent it and listed it as one of his "*Species non satis notae.*" I have already (BOT. GAZ. **76**:92. 1923) shown that HOOKER and ARNOTT (HOOKER etc. Jour. Bot. **3**:319. 1841) equated it with *Thelesperma scabiosoides* Less. This equation was given again by BAKER (Mart. Fl. Brasil. 6^{III}:249. 1884), and in 1898 OTTO KUNTZE, evidently following BAKER'S treatment, created the name *Thelesperma megapotamicum* for *T. scabiosoides* Less. The Berlin Herbarium contains four SELLO sheets of *Thelesperma scabiosoides* Less. While these were assumed by me in a former paper (*l.c.*), in view of KUNTZE, BAKER, and HOOKER and ARNOTT'S treatments, to have been part of the original collection of *Bidens megapotamica* Spreng., it must be noted that none of them bears the name *B. megapotamica* Spreng. This suspicious fact led me in 1924 to a careful search among the Berlin Herbarium specimens, and later through those at the British Museum of Natural History and at Kew. In none of these places was a SELLO specimen found with the name *Bidens megapotamica* Spreng. accompanying it. Recently, however, through the great kindness of Dr. JOHN BRIQUET, in charge of the Delessert Herbarium in Geneva, I have been lent many hundreds of additional sheets of material. Among these is a specimen such as I had hoped to find at Kew. It bears the label "*Brasilia Sello,*" and on the sheet, in badly faded writing, says "*Bidens megapotamica* Spreng." It is mounted on ribbed paper (water-marked "*J. Whatman* 1829," the paper and style of mounting being

in the regulation Kew style of earlier years). It originally belonged to Lambert's Herbarium, whence it was purchased by DELESSERT (*fide* Briquet *in litt.*), and so may have been seen, although misinterpreted, by HOOKER and ARNOTT in England. It is the *Isostigma peucedanifolium* of Lessing. Its heads are somewhat past the flowering stage, and, having lost their rays, appear discoid. SPRENGEL's description is seen to apply to this plant much better than to plants of *Thelesperma scabiosoides* Less.: "B. foliis omnibus 2-pinnatifidis linearifiliformibus glabris, floribus subgeminis pedunculatis erectis discoideis, involucro colorato anthodium aequante." The term "floribus subgeminis" apparently is based upon the tendency of the full-sized heads to sag somewhat on both sides of the peduncle, when pressed flat upon the sheet, and to flare outward at the top as if possessed with a tendency to become more or less cleft or double later on.[9]

10. ISOSTIGMA RIEDELII Schz. Bip. *ex* Baker in MARTIUS Fl. Brasil. 6[III]:240. 1884; also in Ind. Kew. 2:1240. 1893; *cf.* CHODAT, Bull. Herb. Boiss. ser. II. 2:394. 1902; *ibid.* 3:725. 1903.[10] *I. speciosum* var. *Riedelii* (Schz. Bip. *ex* Bak.) Baker, *l.c.*—Pl. XXIII, figs. *i, j, k, l, m, u, x, y.*—Herba erecta, virgata, 8–13 dm. alta, caulibus e rhizomate lignoso interdum etiam 5 cm. crasso ortis. Folia principalia numerosa, ad basim caulis subnudi (pro pedunculo) confertissima, maxime elongata, pallida, nervis parallelis perspicue striata

[9] I have seen no SELLO sheets with the original label of *Tragoceras peucedanifolium* Spreng. SPRENGEL's description was meager and unsatisfactory. He cited no herbarium, nor did LESSING. Yet LESSING's retention of SPRENGEL's trivial name *peucedanifolium* shifts LESSING's type basis back, of course, to whatever SELLO plant SPRENGEL may have studied. In this case, it is doubly reassuring to trace the species back to the first trivial name *megapotamicum* and find an undoubtedly authentic plant to represent it.

[10] BAKER (*l.c.*) says, "Var. *B. Riedelii* (Schultz Bip. *in sched.* Riedel, *s. sp.*)." The *Index Kewensis* published the name more definitely as "*Isostigma Riedelii* Schz. Bip. *ex* Baker," in harmony with its general procedure in somewhat comparable cases. Thus, as an example, for *Glossogyne brasiliensis* Gardn., BENTHAM and HOOKER (Gen. Pl. 2:389. 1876) merely stated that it should go to *Isostigma*. Whereupon the *Index Kewensis* published the name suggested, but not printed, by BENTHAM and HOOKER, namely, *Isostigma brasiliense* (Gardn.) B. and H. (It is significant, however, of the *Index Kewensis* authors' own misgivings as to the absolute validity of such a step that, in the corresponding case of *Bidens purpurea* DC., listed previously to the *Index Kewensis* as *Cosmos purpureus* [DC.] B. and H. by HEMSLEY [Biol. Centr. Amer. Bot. 2:200. 1881], the *Index Kewensis* credited the name's publication not to "BENTHAM and HOOKER," but to "BENTHAM and HOOKER . . . . *ex* HEMSLEY"). CHODAT (*l.c.*) assumed credit for making the specific name *I. Riedelii*, and several botanists have followed him. But clearly, regardless of the merits of its procedure, the *Index Kewensis* must be regarded as having removed any technical shortcomings in connection with the name *I. Riedelii*, and as having given it effective publication years before CHODAT'S treatment.

simplicia, integra vel apice saepe plus minusve profunde trifida, 2.5–4 dm. longa, ultima parte (dimidia-quarta) curvata, linearia et infra sensim angustata, plerumque 4–6.5 mm. lata, residua parte (pro petiolo) angustiora (1–2 mm. lata) et crassiora. Capitula magna, speciosa, solitaria, radiata, pansa ad anthesin 5–6 cm. lata et 1.7–2 cm. alta, pedunculis fere usque ad basim caulium decurrentibus, foliis reductis multis (saepe sub capitulo perspicue numerosis) per omnem partem praeditis. Involucrum campanulatum, bracteis tergo minute glanduloso-scabridis, exterioribus circ. 25 imperfecte biserialibus, linearibus, apice acutis, margine obsolete ciliato saepe albis, usque ad 13 mm. longis, interioribus lanceolatis, margine scariosis, saepe brunneo-purpurascentibus, 1.2–1.5 cm. longis. Flores purpurascentes; ligulati plerumque circ. 12–15 (rarius usque ad 30–32) ligula lineares, intus plus minusve albido-argentei, apice minute sed acriter 2 vel 3 dentulis denticulati, 2–2.5 cm. longi; paleis anguste linearibus, ad apicem purpureis, achaenia matura perspicue superantibus. Achaenia late linearia, obcompressa, striata, glaberrima, nitida, corpore 1.3–1.5 cm. longa et 2–2.5 mm. lata, faciebus atra vel flavido-atra, marginibus anguste alata, alis integerrimis, flavidis, in aristulas binas laevissimas, obscuras vel usque ad 1.5 mm. longas excurrentibus.

Type specimen: *L. Riedel*, in dry fields along the banks of the Rio Pardo, Brazil (Herb. Kew).

Distribution: Paraguay and southern Brazil.

Specimens examined: *K. Fiebrig* 4755, among bushes, dry field at edge of forest, Villa Lana, between the Rio Apa and the Rio Aquidaban, northern Paraguay, January, 1909 (Herb. Berl.; Herb. Deless.; Herb. Gray); *Dr. E. Hassler* 567, fields, Serra dos Esperanza, Sierra de Amambay, Paraguay (Herb. Hassl. in Herb. Deless.); *idem* 5978, on high plateau near the Rio Tapiraguay, Paraguay, December, 1898 (Herb. Hassl. in Herb. Deless.); *idem* 8047, in region of the upper part of the Rio Apa, northern Paraguay, November, 1901 (Herb. Berl.; Herb. Gray; Herb. Kew); *idem* 9238, in the vicinity of Caaguazú, Paraguay, March, 1905 (Herb. Berl.); *L. Riedel*, Brazil (Herb. Kew, type; Herb. Gray); *T. Rojas* (*Dr. E. Hassler distrib.*) 9927, on plateau and slopes, "Sierra de Amambay," Paraguay, December, 1907 (Herb. Berl.); *idem* 10567, *eodem loco*, August, 1907 (Herb. Berl.).

A species with foliar habit much like that of *I. simplicifolium*, although the leaves probably average longer and perhaps are more furcate at the apex. The robust peduncle with its many bracts near and at the apex, also the normal (not dwarfed) rays indicate, however, a close affinity with *I. speciosum*.

11. ISOSTIGMA SPECIOSUM Less., Linnaea **6**:515. 1831; *I. peucedanifolium* var. *genuinum* f. *discoideum* Hassl. *et* f. *radiatum* Hassl., Fedde Rep. Spec. Nov. **14**:277. 1916.[11]—Pl. XXIV, figs. *d*, *e*.—Herba erecta, e rhizomate lignoso, 6–10 vel etiam (fide Hassl. in herb.) usque ad 25 dm. alta, caulibus simplicibus, caespitosis, apice incrassatis et valde squamosis, squamulis linearibus, acutis vel subobtusis, in foliola involucri transeuntibus. Folia ad basim caulis (pedunculi) dense rosulata, petiolata petiolis plerumque usque ad 2 (rariter usque ad 2.7) dm. longis, petiolo adjecto 1.5–3.5 dm. longa, crassa, profunde 3–4-furcata, segmentis petiolo similibus, adscendentibus, subulatis, rigidis, saepe trigona apice acutis, 5–13 cm. longis et 0.9–1.2 mm. latis. Capitula solitaria, magna speciosaque, radiata, pansa ad anthesin 4.5–5.5 cm. lata et 1.5–2 cm. alta, demum cum paleis achaeniisque maturis circ. 2.3 cm. alta. Involucri campanulati bracteae exteriores e caulis superioris (pro pedunculo) squamulis non acriter distinctae, lineares, acutae, adpressae, nunc omnino viridulae et usque ad 1.3 cm. longae, nunc plus minusve albo-marginatae et in bracteas interiores lanceolatas, lateraliter diaphanas, usque ad 2 cm. longas transeuntes. Flores (ex Fiebrig odore melli similes) ligulati circ. 15, extra atro-purpurei intus albido-argentei, ligula lineares, apice plus minusve 3-dentati, 2–2.5 cm. longi; paleis linearibus, achaenia matura facile superantibus. Achaenia oblongo-linearia, obcompresssa, atro-fusca, glabra, corpore circ. 1.6–1.8 cm. longa et circ. 2 mm. lata, anguste alata alis in aristas binas, patulas, laevissimas, obsoletas vel usque ad 1.2 mm. longas excurrentibus.

Type specimen: *Sello*, Brazil[12] (Herb. Berl.).

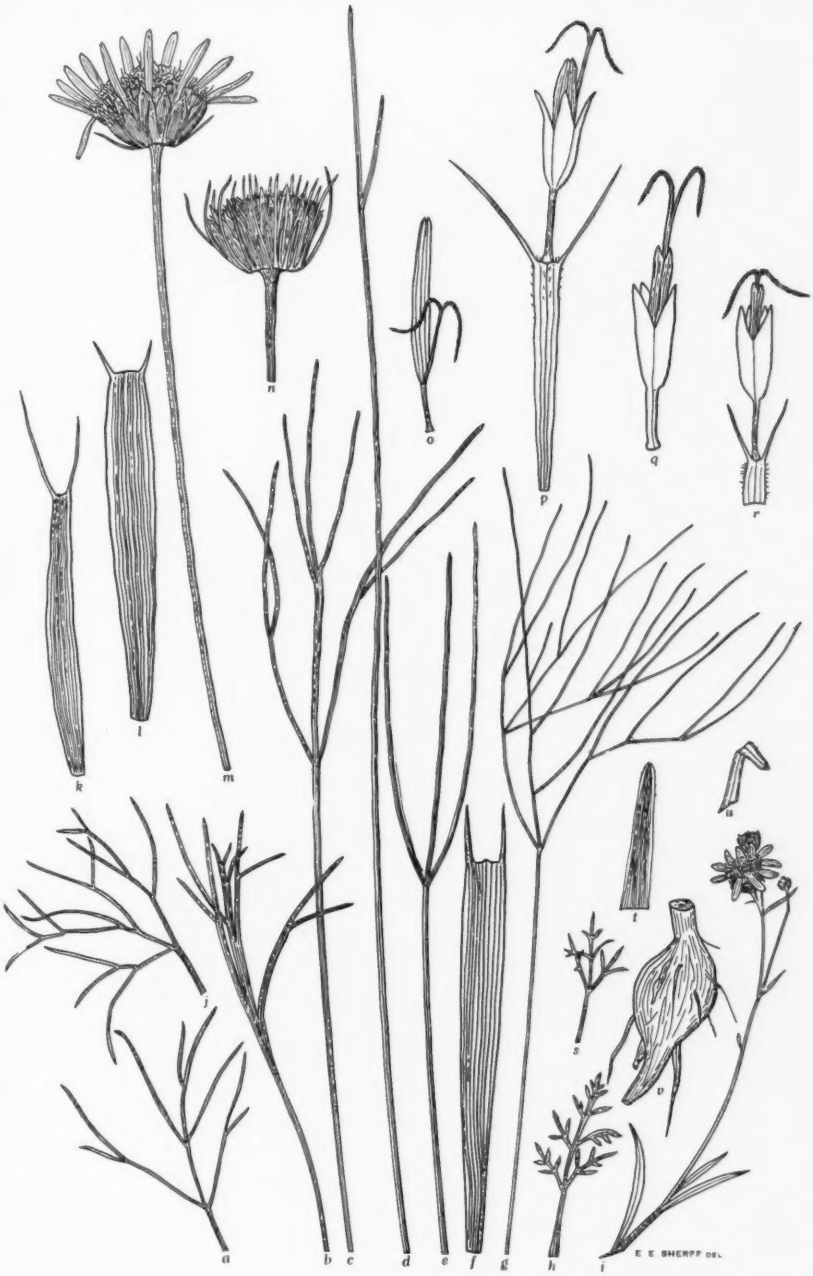
Distribution: Paraguay and southern Brazil.

Specimens examined: *P. Dusen* 4345, in field, in vicinity of Ponta Grossa,

[11] HASSLER'S var. *genuinum* itself, described (*l.c.*) "e specimine typico Herb. Prodromi," doubtless must be referred to *I. speciosum*. His description ("pedunculis foliis reductis linearibus ad 1, 5 cm. longis 1/4 mm. latis alternis-±numerosis ornatis. . . . .") characterizes *I. speciosum* very well.

[12] LESSING (*l.c.*) cited no particular number of SELLO'S. I have before me the six sheets of SELLO'S original material belonging to the Berlin Herbarium. There are three sheets without number and, of the remaining three, one each bears SELLO'S number 243, 1112, and 1723. All six lack the locality, other than Brasilia. The sheet with no. 1723 bears primarily *I. megapotamicum* and has one specimen of *I. speciosum* merely through careless admixture.

SHERFF on ISOSTIGMA



SHERFF on ISOSTIGMA

Paraná, Brazil, March 7, 1904 (Herb. Berl.);[13] *Sello* 243, 1112, 1723 *et sine num.* (type material, 6 sheets in Herb. Berl.).

HASSLER based his *I. peucedanifolium* var. *genuinum* f. *discoideum* upon *Fiebrig* 810, in Herb. Hassl. The five specimens of *Fiebrig* 810 examined by me are in no way to be distinguished from ordinary *I. speciosum*. Several of the heads are well developed and have normal rays. His *I. peucedanifolium* var. *genuinum* f. *radiatum* likewise is purely *I. speciosum*, and so also, doubtless, is his var. *genuinum* itself.

CHICAGO NORMAL COLLEGE
CHICAGO, ILL.

EXPLANATION OF PLATES XXIII, XXIV

PLATE XXIII

Isostigma acaule: a, b, leaves, ×0.54 (type in Herb. Kew); *I. simplicifolium: c, d, e, f*, leaves, ×0.54; *v*, ray floret, ×4.82; *w*, disk floret, ×4.82 (*c, e, f, v, w* from *Sello* 873, type, in Herb. Berl.; *d* from *Claussen*, Minas Geraes, in Herb. Kew); *I. scorzoneraefolium: g, h*, leaves, ×0.54 (from *Riedel*, type of *I. glycinaefolium* in Herb. Kew); *I. Riedelii: i, j, k, l, m*, leaves, ×0.54; *u*, head with upper portion of peduncle, ×0.54; *x*, ray floret, ×3.75; *y*, disk floret, ×3.75 (*i, j* from *Hassler* 9238 in Herb. Berl.; *k, l, m, idem* 10567 in Herb. Berl.; *u, x, y, idem* 8047 in Herb. Berl.); *I. brasiliense: n, o, p, q, r, s, t*, leaves, ×0.54 (*n, o* from *Riedel*, type of *I. stellatum* in Herb. Kew; *p, q, r* from *Glaziou* 21557 in Herb. Berl.; *s* from *Gardner* 4253, type in Herb. Kew; *t, anon.* 98 in Herb. Deless.).

PLATE XXIV

Isostigma dissitifolium: a, j, leaves, ×0.58; *f*, achene, ×0.58 (*a* from *Balansa* 907, type in Herb. Kew; *j, f* from *Fiebrig* 450 in Herb. Berl.); *I. crithmifiolum: b, c*, leaves, ×0.58 (from *Sello* in Herb. Berl.); *I. speciosum: d, e*, leaves, ×0.58 (from *Sello* in Herb. Berl.); *I. megapotamicum: g*, leaf, ×0.58; *k, l*, achenes, ×3.5; *m, n*, flowering and fruiting heads, ×0.58; *v*, ligneous base of stem, ×0.58 (*g* from *Sello* in Herb. Deless.; *k* from *Hassler* 10366 in Herb. Hassl. in Herb. Deless.; *l, m* from *Sello, n, v* from *Sello* 1723, in Herb. Berl.); *I. Hoffmannii: h, s*, leaves, ×0.58; *p, q*, disk florets, ×4.65 (*h* from *Lorentz* in Herb. Berl.; *p, q, s* from *Kuntze*, type sheets in Herb. Berl.); *I. Herzogii: i*, entire type fragment, ×0.58; *o*, ray floret, ×4.65; *r*, disk floret, ×4.65; *t*, interior involucral bract, ×4.65; *u*, exterior involucral bract, ×4.65 (all from *Herzog* 617 in Herb. Hassl. in Herb. Deless.).

[13] *K. Fiebrig* 810, dry elevation at edge of valley, etc., Cerros de Tobati, Paraguay, January 31, 1903 (Herb. Berl., 2 sheets; Herb. Field Mus.; Herb. Gray; Herb. Hassl. in Herb. Deless., type of *I. peucedanifolium* var. *genuinum* f. *discoideum* Hassl.); *Hassler* 5586, on high plateau and slopes, Sierra de Maracayú, Paraguay, December, 1898 (Herb. Hassl. in Herb. Deless., type of *I. peucedanifolium* var. *genuinum* f. *radiatum* Hassl.) *idem* 9238, in dry fields near Caaguazu, Paraguay, March, 1905 (Herb. Hassl. in Herb. Deless.).

PENETRATION PHENOMENA AND FACULTATIVE PARASITISM IN ALTERNARIA, DIPLODIA, AND OTHER FUNGI[1]

PAUL A. YOUNG

(WITH PLATES XXV–XXVII)

Introduction

Between the extremes represented by obligate parasites and obligate saprophytes are numerous facultative parasites which attack other organisms with varying degrees of virulence. Most species of *Alternaria* are facultative parasites. This problem was undertaken to determine the penetration phenomena and experimental host ranges of many dematiaceous and some other fungi. Eighty-eight isolations of fungi and two of bacteria were used in the random inoculations of seventy-eight species and varieties of flowering plants.

Some dematiaceous fungi, *Diplodia*, *Cephalosporium*, and *Colletotrichum*, induced wheat coleoptiles to produce thickenings in the cell walls. The term "callosity" is used to designate these local additions to host cell walls caused by stimulation by a penetrating fungus. The illustrations of the kinds of callosities define them best (figs. 1, 2, 5, 6, 8, 9, 11, 16, 21, 22, 24, 26, 27, 30, 32, 34, 36*A*, 37, 38).

With few exceptions, each callosity incloses a prominent structure resembling a brightly shining dot or slender, angularly irregular line which has been termed the "penetration hypha" (figs. 1, 2, 4, 5, 6, 7, 9, 10, 11, 13, 16, 20, 21, 24, 26, 30, 34, 37, 38). Many direct connections between appressoria and callosities were seen.

The appressoria of *Alternaria* consist of enlarged tips of hyphae

[1] Abstract of a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree at the University of Illinois. The cross inoculations will be considered in greater detail in another article.

The author wishes to thank Dr. F. L. STEVENS for proposing the problem and for numerous helpful suggestions. Thanks are also due to Drs. WILLIAM TRELEASE, W. B. MCDOUGALL, C. F. HOTTES, Mr. L. R. TEHON, and Mr. A. L. HAFENRICHTER of the Botany Department of the University of Illinois, and all others who have aided in the work on this problem.

or their branches; sometimes of enlarged cells in the hyphae (figs. 7, 13, 16, 20, 22, 24, 27). According to BÜSGEN (3), formation of appressoria occurs in many fungi, and is mainly due to stimuli resulting from contact of the germ tubes with solid bodies.

"Vertical cell walls" refer to the cell walls which appeared to be vertical when the surfaces of strips of epidermis were microscopically examined. Unless otherwise stated, wheat means Red Wave wheat. Most of the inoculations in tubes were made on seedling stems; most of the greenhouse inoculations were made on leaves.

Methods

Fungi were grown on corn-meal agar. Seeds were surface sterilized with a 3 per cent solution of Chloramine-T or a 0.5–1 per cent solution of Uspulun. Seeds were transferred directly from the disinfectants to the wet filter paper in the bottoms of large, autoclaved dishes of the Petri dish type. The dishes were then kept in a dark incubator at 25° C., until the plumules of the seedlings were 1–4 cm. long. Usually within 3–5 days, plumules of wheat, rye, barley, oats, sorghum, and broom corn reached a length of 1–3 cm. without the coleoptiles being ruptured. Uninjured seedlings were inoculated and grown according to the method used by STEVENS (17). Only aseptic seedlings were used. Spores or agar bearing mycelium of fungi were placed on the epidermis of the stems of aseptic seedlings, which were then rolled in wet, autoclaved rags and placed in large tubes. Seedlings in tubes were grown in the light at about 25° C. for 2–14 days after inoculation. Check seedlings remained aseptic for a week or often longer. Wheat seedlings began to show symptoms of drought and starvation injury after they had been in the tubes about 10 days.

Within 2–14 (usually 3–8) days after inoculation, seedlings were examined by the following method. The point of a needle was inserted under the outer 1–3 layers of epidermal cells of an inoculated region. In the case of wheat, a free end was grasped with forceps and a thin strip pulled off. In the case of cabbage, it was necessary to slide the needle under the strip to be removed before the ends were broken loose. Strips were usually stained with acid fuchsin or Congo red, and mounted in glycerine on slides, which were then sealed with gold size. Microtome sections were stained with Pianeze III B.

Spores or agar bearing mycelium of fungi were placed in drops of water on uninjured leaves of greenhouse plants, which were then covered for usually 3 days with bell jars lined with wet filter paper. The bell jars were covered with heavy burlap sacks to prevent sun scalding. Spores usually floated on the drops of water, and were often arranged in rings marking the margins of the layers of water when these drops were covered with cover glasses.

Potted plants were inoculated and placed in large glass cases into which water was sprayed for three days. Such inoculations usually did not result in infections.

Host reactions to cross inoculations

Nearly all the details from 77 tables of cross inoculations are omitted here; some of them will be given in another article. Penetration phenomena are grouped according to hosts.

Triticum aestivum.—All species of *Alternaria*, *Acrothecium*, and *Helminthosporium* placed on wheat coleoptiles caused them to form callosities; most of them caused brown cells. They usually altered regions in cell walls, which appeared as disks or rings when the strips were stained. *Diplodia zeae* (Schw.) Lev., *Cephalosporium acremonium* Corda, and *Colletotrichum nigrum* E. & H. also caused wheat coleoptiles to form callosities.

Infection of wheat coleoptiles by *Alternaria* and *Helminthosporium* results in similar penetration phenomena, so reference is made to the illustrated account by STEVENS (17) of *Helminthosporium* penetration. MANGIN (10) seems to deserve credit for first illustrating and describing the bodies called callosities; he reported them in wheat infected with *Septoria*. FRON (6) illustrated callosities caused by *Leptosphaeria* in wheat. Comparable bodies were described by RAVN (14), DASTUR (4), BREFELD (2), and WOLFF (19). NEGER (12), HIGGINS (8), and SMITH (16) say that hosts form sheaths around the penetrating hyphae of the Erysiphaceae and Coccomyces.

Spores of *Alternaria* and *Helminthosporium* were germinated in water under cover glasses on slides, and stained with dilute gentian violet. Some of the germ tubes in each case showed sheaths. Contact of the germ tubes of these fungi with glass did not cause them to form appressoria.

Examination of wheat coleoptiles within 48 hours after inoculation with *Alternaria* often shows germinated spores bearing appressoria and pegs which have caused the host to produce small callosities; some brown cells may be visible at this time. Stains show that the epidermis near the points of incipient penetration has been greatly altered. The magnitude of the symptoms and accompanying fungous development increases, so that strips of epidermis examined a week after inoculation generally exhibit most of the effects described later; strips removed two weeks after inoculation often show no more than these.

On the lower sides of appressoria appear slender outgrowths (penetration hyphae) which enter the cuticle of the epidermal cells. Wheat cells react to this penetration by the formation of callosities around the penetration hyphae. Each callosity incloses a prominent body which resembles a brightly shining dot or an angularly irregular line which is the penetration hypha. In most cases the callosities increase in size more rapidly than the penetration hyphae, and so continue to inclose them. In many cases, however, the penetration hyphae project from the callosities and cross 1–3 host cells (figs. 2, 9, 13).

Wheat callosities are usually hemispherical in outline and have distinct margins; they are often elongate and sometimes radiate, toothed, or irregular in shape (figs. 1, 2, 5, 6, 8, 34, 38). Callosities often formed dense fields and were frequently packed together; appressoria were usually attached to most callosities, unless torn away. *Diplodia* and *Cephalosporium* induced the formation of circular callosities which were smaller and elongate callosities which were narrower than those caused by *Alternaria;* they were usually more numerous in a unit area. Numerous cases of appressoria attached to callosities by penetration hyphae were seen (figs. 13, 16). Internal hyphae often sent batteries of penetration hyphae into adjacent cell walls and induced many callosities to form; one internal hypha of *Helminthosporium gramineum* sent out a row of 35 penetration hyphae with many more projecting from neighboring internal hyphae. Internal hyphae of *Alternaria* were seen to send out smaller numbers of penetration hyphae. No *Alternaria* penetration hypha was observed to become an internal hypha.

In areas of penetration of wheat and other hosts, the cell walls

were much thickened and exhibited prominent middle lamellae (figs. 1, 2, 3*E*, 4, 5, 9, 10, 12, 13, 16, 24, 28). *Alternaria* species from asparagus and rose produced hyphae in pockets in swollen vertical cell walls of wheat (fig. 12). Penetration hyphae of *Alternaria* usually entered vertical cell walls; ADERHOLD (1) and STEVENS described this phenomenon in penetration by other fungi.

Most of the wheat strips were stained with acid fuchsin or Congo red. Brilliant red rings or disks around the points of incipient penetration showed that the cell walls had been greatly altered at these points.

Yellow, auto-stained disks were seen in the cell walls of one wheat coleoptile infected with *Alternaria;* a gray, auto-stained disk was seen in a coleoptile infected with *Acrothecium*. KLEBAHN (9) described auto-stained disks in tomato stems infected with *Didymella*. Stained disks of altered cell wall material were illustrated by MANGIN (10) and STEVENS (17). EDSON (5) says that the first visible indication of alteration of beet cell walls infected with *Phoma* is a change in their reaction toward the stain.

The red stained rings in wheat coleoptiles caused by *Alternaria*, *Helminthosporium*, *Cephalosporium*, or *Colletotrichum* may be divided into four regions (fig. 4): (1) the callosity itself is usually unstained; (2) the adjacent region is lightly or not at all stained (deeply stained in red disks); (3) a broad, red band surrounds the first two regions and forms the ring; and (4) the unstained, normal host cell walls lie outside the ring. Perhaps some chemical diffused from the penetration hypha in the callosity into the cell wall and caused differential chemical alteration of the wall in different places. It is also possible, although it seems less likely, that physical stimuli from the penetration hypha may have induced physical alteration in the cell wall such as porosity, and thus affected the retention of the stain in some places. Microchemical proof is lacking. These areas, when stained with Pianeze III B, exhibit green centers surrounded by purple borders (fig. 11). A strip of wheat coleoptile infected with *H. gramineum* exhibited blue-purple disks when stained with gentian violet.

Chloro-iodide of zinc (made by dissolving Merck's zinc chloro-iodide powder in a volume of water smaller than itself and adding

an excess of iodine crystals to make a red solution) caused brilliant, yellow-brown disks to appear around points of incipient penetration in wheat coleoptiles infected with *Alternaria;* the callosities became dark yellow-brown, but these colors faded within a few days. The inner lamellae of swollen walls became brown when treated in this way. Treatment of infected wheat strips with phloroglucin and hydrochloric acid caused bright red rings to appear around the incipient infection points; the rings soon faded.

Callosities turned brown when treated with nitric acid. Boiling in water did not affect them. Wheat strips were soaked in acetone and others in ether for 5 days without apparent changes in the contained callosities. Wheat callosities were not discolored by iodine dissolved in potassium iodide solution, nor with this treatment followed by concentrated sulphuric acid. These microchemical and staining tests were too incomplete to justify conclusions concerning the chemical nature of callosities, or the nature of the alteration of the cell walls around infection points.

Brown cells occur singly or in small groups in wheat coleoptiles infected with *Alternaria;* browned regions of cells are often large enough to be macroscopically visible as brown streaks. Coleoptiles often become entirely brown, rotten, and bear aerial mycelium; only rarely can thin strips be removed from such coleoptiles. *Helminthosporium* from barley produced new sporophores and spores on wheat coleoptiles in tubes within 4 days after inoculation. Most of the wheat strips were mounted in glycerine. Normal cells were badly plasmolyzed, while brown cells apparently were unaffected by this treatment.

It was tentatively assumed that *Alternaria* and *Helminthosporium* possessed chemicals which altered wheat cell walls and induced callosities to form. To try to determine whether or not such chemicals could enter wheat coleoptiles in the absence of mycelium, *Alternaria* (*Macrosporium avenae* Oud.?) and *Helminthosporium gramineum* were grown in nutrient broth of the following formula: 20 gm. of Difco bacto-beef, 1 gm. of $FeSO_4.NH_2SO_4.6H_2O$, 10 gm. of cane sugar, 7 drops of lactic acid, and 400 cc. of distilled water. A second formula was used later for growing the *Alternaria:* 10 gm. of bacto-beef, 3 drops of lactic acid, and 250 cc. of distilled water.

The mixtures were boiled for a few minutes, filtered, placed in Kolle flasks, and autoclaved. After the fungi had grown on these media for 2–4 weeks, the liquids remaining in the flasks were aseptically filtered through two layers of filter paper. The cloths surrounding aseptic wheat seedlings were saturated with the filtrates and the plants grown in tubes for a week. Liquid pressed from the sectioned mycelial mat of *Alternaria* was also used in this way. In no case did any of the wheat coleoptiles exhibit callosities or red stained rings. These repeated results with no exceptions lead to the conclusions that such penetration phenomena are not due to chemicals from the fungi, or that such chemicals do not penetrate wheat coleoptiles in the absence of mycelium.

It is generally assumed that mechanical injuries of plants cause discolorations. This assumption was proved to be true in the cases of wheat, sorghum, and broom corn. Aseptic seedlings of these plants were repeatedly wounded with the point of a sterile needle and the plants grown in sterile tubes for several days after wounding. Strips from the wounded coleoptiles showed yellow discoloration in wheat, and red-brown discoloration in sorghum and broom corn, in the cells surrounding the wounds (fig. 15). No callosities, autostained disks, or red rings appeared. Since fungi and bacteria were absent, these results prove that mechanical injuries (including breaks) induce these seedling plants to produce intense discolorations in the injured regions. PARKIN (13) described discoloration of *Jacobina*.

Diplodia zeae induced wheat coleoptiles to produce very dense fields of yellow, elongate and circular callosities (fig. 34). Numerous long penetration hyphae occurred. A few elongate callosities were broken out of some of the ruptured wheat cells in an inverted strip. These observations show that elongate callosities are attached by one or both ends; that the centers of such callosities may be free from the cell walls; and that elongate callosities sometimes project down into cell cavities.

Coleoptiles of wheat badly infected with this fungus showed intense browning of entire coleoptiles which bore abundant, white aerial mycelium on the brown regions. Abundant internal mycelium and some radiate callosities were present. Usually 100 per cent in-

fections occurred with this fungus in the five series of inoculations with it.

Cephalosporium acremonium induced wheat coleoptiles to produce great numbers of usually hyaline callosities, which resembled those caused by *Diplodia* in shape and density of fields; some appressoria were seen in contact with callosities. Many inoculations of wheat in the five series failed to result in infections; infected coleoptiles were usually slightly or not at all discolored. Many red stained rings and disks, and some large, complex callosities were seen; Congo red was retained around the penetration hyphae in the callosities in some cases. Many long, freely projecting penetration hyphae were observed. One slide showed two small spots, each of which bore hundreds of penetration hyphae. In one case the spot appeared to be a mycelial mass, perhaps a complex appresorium, which was thickly studded with pegs, and from which a row of many penetration hyphae projected and caused callosities in an adjacent cell wall. Much internal mycelium was seen in one strip; some ladder mycelium passed from cell to cell in a manner resembling that shown in fig. 36. Many brown cells seemed to be nearly full of mycelium. REDDY and HOLBERT (**15**) discuss this fungus.

Colletotrichum nigrum induced the formation of callosities and altered regions in wheat cell walls. These phenomena often occurred under appressoria (fig. 10). One of the many very large red disks exhibited three appressoria with many radiate callosities in the cells below them. Other wheat strips showed great numbers of red rings, some of which contained minute, circular callosities; each of the callosities and many of the red rings exhibited brightly shining dots representing the penetration hyphae. The strips in another series of inoculations were more heavily infected; some of them bore abundant internal mycelium and superficial acervulae containing many spores. HASSELBRING (**7**) described the appressoria of anthracnose fungi. Many inoculations of wheat with this fungus failed.

Acrothecium and *Macrosporium cucumerinum* E. & E. induced wheat coleoptiles to form callosities; the penetration phenomena were similar to those caused by *Alternaria*. MITRA (**11**) says that *Acrothecium* penetrated *Pennisetum* cuticles.

Internal hyphae of *Alternaria* in wheat were usually brown

(sometimes hyaline), and consisted of single filaments, or more often of bands or ribbons, probably formed by the lateral fusion of two or more hyphae. The diameters of the cells of internal hyphae were often greater than those of normal superficial hyphae. Longitudinal sections of seedling wheat stems infected with *Alternaria* (*Macrosporium iridis* C. & E.) showed compact masses of hyphae deep in the host tissues (fig. 3). Callosities occurred in cell walls below the internal mycelium of *Alternaria* in one coleoptile. *Alternaria* sp. from asparagus produced broad bands of brown, internal hyphae (fig. 33*A*).

The superficial hyphae of three species of *Alternaria* became aggregated into compact, brown, fan-shaped masses on wheat and pumpkin seedling stems. STEVENS pictured similar masses of *Helminthosporium* mycelium. The smaller aggregations of superficial hyphae formed tangled masses.

Alternaria sp. from apple surface rot produced very long penetration hyphae in wheat (figs. 2, 13). Internal mycelium of *Alternaria* sp. from rose passed from cell to cell in a seedling wheat stem like the mycelium shown in fig. 36. One germ tube of *Alternaria brassicae* (Berk.) Sacc. f. *microspora* P. Brun. caused eight callosities in a wheat coleoptile like those shown in fig. 5.

No infections resulted when wheat coleoptiles were inoculated with species of *Penicillium*, *Aspergillus*, *Sterigmatocystis*, *Syncephalastrum*, *Phytophthora*, *Saprolegnia*, *Cunninghamella*, *Botrytis*, *Sclerotinia*, *Cephalothecium*, *Fusarium*, *Epicoccum*, *Thielavia*, *Thielaviopsis*, *Pestalozzia*, *Chaetomium*, and two isolations of white-colony bacteria. Species of *Alternaria* from wheat, corn, and cabbage did not cause wheat leaf spots; *Alternaria* (*Macrosporium avenae*) caused a few wheat leaf spots. *Helminthosporium gramineum* caused green infection spots to appear in wheat leaves which had turned yellow under the bell jar (figs. 35*A*, *B*).

Avena sativa, *Secale cereale*, *Hordeum vulgare*, *Zea mays* var. *everta*, and *Z. mays* var. *indentata*.—The reactions of oats, rye, barley, pop corn, and field corn to penetration and infection by *Alternaria* and *Helminthosporium* were similar to wheat reactions. Five isolations of *Alternaria* induced callosities and produced internal hyphae in oat coleoptiles; three species caused the formation

of large, prominent, yellow, auto-stained disks around incipient infection points in oats. Four isolations of *Alternaria* caused oat leaf spots. *Helminthosporium gramineum* induced the formation of three callosities and caused spots in oat leaves. Regions in a seedling stem of field corn infected with this fungus exhibited one red disk, circular, elongate, and complex callosities, some internal hyphae, some appressoria, and superficial fan-mycelium.

Cephalosporium acremonium caused callosities in field corn leaves. This fungus, *Macrosporium cucumerinum*, *Diplodia zeae*, six isolations of *Alternaria*, and two species of *Helminthosporium* caused corn leaf spots. Seven isolations of *Alternaria* caused pop corn coleoptiles to form callosities; five isolations of *Alternaria* caused leaf spots.

Alternaria sp. from wheat caused some callosities in rye. *Helminthosporium gramineum* caused five green spots in rye leaves that had turned yellow under the bell jar. Two species of *Alternaria* caused callosities in barley coleoptiles.

Holcus sorghum.—Infections of sorghum and broom corn seedlings by *Alternaria*, and also purely mechanical injuries, characteristically resulted in the appearance of deep red-brown discoloration of the injured regions (fig. 15). No callosities or auto-stained disks appeared when fungi were not present. The red-brown pigment was produced abundantly and often diffused from diseased or mechanically injured coleoptiles and stained the surrounding cloths.

Points of incipient infection of sorghum coleoptiles by *Alternaria* were often marked by red-brown (sometimes gray) auto-stained disks (figs. 14, 20). Such disks were caused by five species of *Alternaria* and one of *Helminthosporium*. Twelve isolations of *Alternaria* caused brown callosities in sorghum coleoptiles; nine isolations produced internal hyphae. Sections of a sorghum stem infected with *Alternaria ribis* Bub. & Ranoj.? exhibited mycelium in the four outer layers of cells. Six species of *Alternaria* caused sorghum leaf spots; two species caused broom corn leaf spots. Four species caused callosities in broom corn coleoptiles.

Brassica oleracea, *B. rapa*, and *Raphanus sativus.*—Eleven isolations of *Alternaria* caused circular, yellow callosities in cabbage seedling stems; appressoria were usually present on the callosities.

Hyphae of *Alternaria* sp. from *Abutilon* occurred in swollen vertical cell walls (figs. 18, 19). Nine isolations of *Alternaria* produced internal hyphae in cabbage cells. Three species of *Alternaria* and *Helminthosporium gramineum* caused cabbage leaf spots; six species of *Alternaria* did not cause leaf spots. *Alternaria* sp. from pepper leaf spot caused brown, auto-stained disks in seedling cabbage stems.

Four species of *Alternaria* caused callosities in radish seedling stems, five species produced internal hyphae, and five species caused radish leaf spots. *Alternaria brassicae microspora* and an undescribed species from wheat seeds caused green spots in radish leaves which had turned yellow (figs. 40, 43). Four species of *Alternaria* caused callosities in turnip seedling stems; two of them produced internal hyphae.

Cucurbita Pepo and *Cucumis sativus*.—Five isolations of *Alternaria* and three of *Helminthosporium* caused callosities in pumpkin seedling stems (figs. 27, 32); three of these fungi caused callosities in pumpkin stem hairs (fig. 29). Three species of *Alternaria* and two of *Helminthosporium* produced internal hyphae. Three species of *Alternaria* did not infect pumpkin seedlings. *Alternaria* sp. from *Abutilon* produced abundant mycelium in pumpkin cotyledons (fig. 33). *Alternaria* sp. from gooseberry caused some callosities in a watermelon seedling stem.

Three species of *Alternaria*, two of *Helminthosporium*, and *Diplodia zeae* caused callosities in muskmelon seedling stems; all except the last produced internal hyphae. Ladder mycelium of *H. gramineum* is shown in fig. 36. *Alternaria* (probably *Macrosporium iridis*) produced mycelium in swollen vertical cell walls (fig. 24).

Glycine max, *Vigna sinensis*, and *Pisum sativum*.—*Alternaria* infection of soy bean seedling stems resulted in the appearance of autostained, yellow, granular, disk-shaped spots surrounding points of incipient infection; they seemed to be made up of aggregations of yellow droplets surrounding penetration hyphae. Most of the penetration hyphae entered vertical cell walls. Callosities which often had partially indefinite margins inclosed many of the penetration hyphae (figs. 21, 30). Perhaps the hypha shown in fig. 28 represents a compound appressorium. Three species of *Alternaria* caused callosities and produced internal hyphae in soy bean stems; many

of the infected cells turned brown. *Helminthosporium gramineum* caused a yellow callosity in a wax bean seedling stem; leaf spots of this host were caused by this fungus and *Phytophthora cactorum*. Three species of *Alternaria* did not infect uninjured bean leaves.

Alternaria from radish leaf spots caused cowpea leaf spots; another *Alternaria* and two species of *Helminthosporium* failed to do so. Hyphae of *Macrosporium iridis* entered a garden pea leaf through the cuticle and also through a stoma; leaf spots appeared later. *Helminthosporium* sp. and two species of *Alternaria* also caused pea leaf spots.

Abutilon Theophrasti.—*Alternaria* sp. from pepper leaf spot caused this host to form small, circular callosities; *Alternaria* sp. from wheat produced internal hyphae.

Allium Cepa.—Onion bulbs were surface-sterilized with chemicals, almost vertically severed with a knife, and spores or mycelium placed on the membranes between the scales, which were then forced back together, bound with sterilized cloth or rubber bands, and the bulbs then stored in autoclaved dishes for 3–8 days. The onions sent out roots and leaves and remained in fairly good condition for a week.

Macrosporium iridis and *Alternaria* (probably *Macrosporium parasiticum* Thüm.) penetrated onion membranes by sending hyphae of normal diameter through swollen vertical cell walls (fig. 31).

Batteries of *M. iridis* mycelium passed through greatly swollen, brown, vertical cell walls. Another isolation of *M. parasiticum* produced abundant internal mycelium.

Five isolations of *Alternaria* and *Macrosporium* caused aggregations of irregular particles to appear as granular, disk-shaped, hyaline or brown spots surrounding points in vertical cell walls of onion bulb membranes (fig. 23); slender penetration hyphae were not seen. Ten isolations of *Alternaria* and also *Diplodia zeae* did not penetrate onion bulb membranes. Four species of *Alternaria* and *Helminthosporium gramineum* failed to infect onion leaves.

Lycopersicon esculentum, Capsicum frutescens, and *Solanum nigrum.*—Callosities induced by eight isolations of *Alternaria* in stems of tomato seedlings were small, circular in outline, yellow, and often consisted of short, swollen regions in vertical cell walls; each callosity inclosed a penetration hypha. Appressoria were usually

present on the callosities. Many of the tomato callosities caused by *Alternaria* sp. from *Abutilon* resembled small, brown, auto-stained disks; this species exhibited one case of stomatal penetration of a tomato seedling (fig. 17). Five species of *Alternaria* produced internal mycelium in tomato stems.

Alternaria from pepper end rot caused prominent pepper leaf spots. Cone-shaped masses of mycelium of *Macrosporium iridis* were seen in sections to penetrate pepper leaves to a depth of 2–4 cells; similar mycelial masses were seen in *Chenopodium album* L. leaves.

Alternaria sp. from tomato end rot infected many hosts, but did not cause leaf spots of pepper or egg plant. *Alternaria* sp. from pepper end rot caused prominent leaf spots in *Datura tatula; Helminthosporium gramineum* also caused leaf spots. *Alternaria* sp. from tomato caused a green leaf spot in a *Solanum nigrum* leaf which had turned yellow under the bell jar; *Alternaria* sp. from pepper end rot caused leaf spots on this host.

Impatiens pallida exhibited leaf spots caused by five isolations of *Alternaria;* aerial mycelium projected from the lower sides of many of the leaf spots.

Besides those here indicated, hundreds of other cross inoculations were made in the laboratory and in the greenhouse.

Discussion

The two hundred new diseases which appeared in the cross inoculations under the conditions described, exhibited symptoms ranging from isolated callosities and scattered brown cells visible only when microscopically examined, to seedling stem rots and large leaf spots. These diseases appeared under conditions very unlike field conditions, so that they are not apt to become serious. Aseptic seedlings in tubes and plants under bell jars were physiologically different from genetically similar plants growing in fields.

A high percentage of the leaf inoculations in the greenhouse did not result in infections. This does not necessarily mean that the hosts were immune in such cases, even though conditions were supposedly very favorable for infection; inoculations were usually not sufficiently numerous and varied to test immunity.

In the cross inoculations, a fungus was said to be the cause of a

leaf or stem spot when diseased lesions appeared below the spores or mycelium used in inoculation and not elsewhere within 1–5 days after inoculation; many isolations and reinoculations were made. In many cases the inoculation work was too incomplete to prove the causal relationships of the fungi below which the spots appeared. Tube inoculations consisted of pure cultures of fungi placed on aseptic seedlings.

The theory has been proposed that the material formed by hosts around penetrating hyphae performs the function of stopping the growth of the penetrating hyphae which they inclose; this seems to be true of most of the callosities seen. The presence of long penetration hyphae projecting from callosities, however, shows that such callosities failed to stop the growth of the penetration hyphae.

Thousands of the slender penetration hyphae of *Alternaria* were examined. Since no case of one of them becoming an internal hypha was observed, perhaps such a phenomenon does not occur in the penetration of wheat coleoptiles by *Alternaria*. It may be that the growth of the penetration hyphae is retarded, or finally stopped, even after they pass beyond the margins of callosities. The presence of many penetration hyphae projecting from internal hyphae of *Alternaria* and *Helminthosporium* is interpreted to mean that the internal hyphae produced the penetration hyphae.

Alternaria and *Helminthosporium* penetrate and infect plants very rapidly in contrast to the slowness with which many fungi of economic importance enter their hosts and cause diseases. In leaves inoculated with *Alternaria* and *Helminthosporium* under bell jars, leaf spots usually appeared within 1–5 days or not at all. Hypophyllous aerial mycelium appeared on many leaf spots within 3 days after inoculation. *Helminthosporium* sp. from barley produced new sporophores and spores on wheat plants in tubes within 4 days, and on its barley leaf spots within 3 days after inoculation. *Helminthosporium gramineum* produced rings of hypophyllous aerial mycelium on its *Impatiens* leaf spots. This fungus and two species of *Alternaria* caused red infection rings below their rings of spores on sorghum leaves (fig. 41).

Some differences in the infections of wheat by *Alternaria* and *Helminthosporium* should be noted. The appearance of great numbers of callosities and red stained rings or disks is characteristic of

infection by *Alternaria*. In contrast to this, *H. gramineum*, for instance, usually causes the appearance of few or no callosities or red stained rings or disks. The appearance of deeper browning of host cells and the presence of more abundant internal mycelium is characteristic of *Helminthosporium* infection of wheat coleoptiles in tube cultures. STEVENS described infection by *Helminthosporium*.

In the inoculation of wheat coleoptiles with *Penicillium* sp., *Botrytis cinerea*, *Cephalothecium roseum*, and *Epicoccum* sp., each of these fungi produced a few simple appressoria without causing the coleoptiles to show any penetration phenomena. Perhaps this demonstration of attempted penetration represents the manner in which parasitism began in fungi which penetrate directly through epidermal cells of host plants. *Alternaria* appressoria often do not produce penetration hyphae which infect cells below them. A majority of the specialized appressoria of *Colletotrichum nigrum* seen on wheat were not associated with infections.

Alternaria penetration of onion bulb membranes was entirely unlike the penetration of wheat coleoptiles. Penetration was by hyphae of normal diameter, which passed through greatly swollen vertical cell walls. The disk-shaped aggregations of irregular granules lacked prominent penetration hyphae; callosities and red stained rings were not seen.

A pumpkin cotyledon reacted to invasion by *Alternaria* sp. from *Abutilon* mycelium by the production of two boundary layers in the lower part of the cotyledon (fig. 33). Subsequent research gave sections showing similar boundary layers in the upper parts of cotyledons in which no mycelium was present. Few of the thirty inoculations of pumpkin cotyledons resulted in infections. Sections of two of the cotyledons showed abundant mycelium in the boundary layers and in cells not cut off by these layers. Such layers occurred under white regions over the midribs of four cotyledons (both of those on two plants); they appeared to cut off part of the palisade and storage tissues. Sections of brown spots which appeared under mycelium did not contain mycelium or show abnormalities.

Internal hyphae of *Alternaria* often assumed the form of ribbons or bands of laterally fused hyphae (figs. 1, 7, 33*A*). These bands were usually brown (sometimes hyaline), and were made up of ir-

regular cells which were often larger than the cells of ordinary superficial mycelium. Compact, fan-shaped masses of brown *Alternaria* and *Helminthosporium* mycelium occurred both in and on wheat coleoptiles. The prominent radiate appearance of such mycelium suggests the radiate mycelium of the Trichopeltaceae and the radiate perithecia of the Microthyriaceae. WOLF (**18**) used such a character in classifying *Diplocarpon*. STEVENS pictured fan mycelium of *Helminthosporium*. It does not seem that radiate mycelium proves relationship to the Microthyriaceae.

Summary

1. Mechanical injury alone caused cells of wheat, sorghum, and broom corn to become markedly discolored near the points of injury; such discolored regions lacked callosities, auto-stained disks, red rings, and other infection phenomena.

2. The two hundred new diseases which resulted from the cross inoculations occurred under conditions very unlike field conditions.

3. All species of *Alternaria* placed on wheat coleoptiles induced the formation of callosities; all except three produced internal hyphae.

4. *Alternaria* infection of wheat coleoptiles is characterized by the formation of callosities by the host, and by the appearance of stained rings or disks around points of incipient infection.

5. *Alternaria* caused callosities to appear in seedling stems of wheat, oats, rye, barley, pop corn, sorghum, broom corn, cabbage, radish, turnip, tomato, *Abutilon*, soy bean, wax bean, watermelon, pumpkin, and muskmelon; *Helminthosporium gramineum* and *Cephalosporium acremonium* caused them to appear in field corn.

6. *Diplodia zeae*, *Cephalosporium acremonium*, *Colletotrichum nigrum*, and *Acrothecium* sp. induced wheat coleoptiles to produce callosities.

7. Detached and broken elongate callosities induced in wheat by *Diplodia* showed that elongate callosities sometimes project down into cell cavities, and that they are attached by one or both ends.

8. Different combinations of hosts and fungi resulted in the formation of different kinds of callosities.

9. Penetration hyphae usually entered vertical cell walls.

10. In tube cultures, epidermal cells of hosts below *Alternaria* inoculum often showed isolated groups or single brown cells; internal hyphae were not seen in most of such cells.

11. *Alternaria* spp. from asparagus and rose produced internal hyphae in swollen vertical cell walls of wheat; similar internal hyphae of *Alternaria* sp. from *Abutilon* occurred in vertical cell walls of cabbage, and some were produced in vertical cell walls of muskmelon by *Alternaria* sp. from iris.

12. *Alternaria* and *Helminthosporium* usually penetrated the hosts here considered within 1–5 days or not at all.

13. Sorghum and broom corn seedling stems infected with *Alternaria* often exhibited prominent red-brown, auto-stained, disk-shaped spots around incipient infection points. Auto-stained spots, yellow or gray in color, were seen in the epidermal cells of wheat, oats, tomato, cabbage, and soy bean seedling stems infected with *Alternaria*.

14. Infected regions of sorghum and broom corn always had a prominent red-brown color. The pigment was produced abundantly, and often diffused out of infected or mechanically injured seedlings and stained the rags surrounding them.

15. Rings of spores of two species of *Alternaria* and one of *Helminthosporium* on sorghum leaves caused red infection rings.

16. Species of *Penicillium*, *Botrytis*, *Cephalothecium*, and *Epicoccum* produced appressoria on wheat coleoptiles, but caused no infections.

17. Species of *Syncephalastrum*, *Saprolegnia*, *Cunninghamella*, *Botrytis*, *Cephalothecium*, *Fusarium*, *Thielavia*, *Thielaviopsis*, *Pestalozzia*, *Chaetomium*, *Aspergillus*, and *Sterigmatocystis* did not penetrate wheat coleoptiles.

18. Two species of *Alternaria* and one of *Helminthosporium* caused callosities to form in pumpkin hairs.

19. Soy bean seedling stems infected with *Alternaria* exhibited yellow, disk-shaped aggregations of droplets or granules at points of incipient infection.

20. Stomatal penetration by *Alternaria* occurred in a pea leaf and a tomato stem; no other cases were seen.

21. *Alternaria* penetration of onion bulb membranes consisted of hyphae of normal diameter passing through swollen vertical cell walls.

22. Since broth filtrates in which *Alternaria* and *Helminthosporium* had grown and liquid pressed from *Alternaria* mycelium caused no callosities or altered spots to appear in wheat coleoptiles, it is concluded that chemicals from these fungi do not cause such reactions, or that such chemicals do not enter wheat coleoptiles in the absence of mycelium.

AGRICULTURAL EXPERIMENT STATION
BOZEMAN, MONT.

LITERATURE CITED

1. ADERHOLD, R., Die Fusicladien unserer Obstbäume. Landw. Jahrb. **25**: 875–914. 1900.
2. BREFELD, O., Untersuchungen aus dem Gesammtgebiete der Mykologie. Die Brandpilze II. **11**: *figs. 2, 3.* 1895.
3. BÜSGEN, M., Über einige Eigenschaften der Keimlinge parasitischer Pilze. Bot. Zeit. **51**:53–72. Taf. III. *figs. 15, 32.* 1893.
4. DASTUR, J. F., The mode of infection by smut in sugar cane. Ann. Botany **34**:391–397. *figs. 8, 9.* 1920.
5. EDSON, H. A., Histological relations of sugar beet seedlings and *Phoma betae.* Jour. Agric. Res. **5**:55–58. *pl. I.* 1915.
6. FRON, M. G., Pied noir des céréals ou maladie piétin. Ann. Sci. Agron. **29**:3–29. *pls. I, II.* 1912.
7. HASSELBRING, H., The appressoria of the anthracnoses. BOT. GAZ. **42**:135–142. 1906.
8. HIGGINS, B. B., Contribution to the life history and physiology of *Cylindrosporium* on stone fruits. Amer. Jour. Bot. **1**:145–173. *pl. XV, fig. 18.* 1914.
9. KLEBAHN, H., Der Pilz der Tomatenstengelkrankheit und seine Schlauchfruchtform. Zeitschr. Pflanzenkr. **31**:1–16. *figs. 1–5.* 1921.
10. MANGIN, L., Sur le *Septoria graminum* Desm., destructeur des feuilles du blé. Bul. Soc. Myc. France **15**:108–126. *pl. VIII;* also 210-239. 1899.
11. MITRA, M., Morphology and parasitism of *Acrothecium penniseti,* n. sp. Mem. Dept. Agric. India (Bot. Ser.) **11**:57–74. *pl. IV.* 1921.
12. NEGER, F. W., Beiträge zur Biologie der Erysipheen. III. Der Parasitismus der Mehltaupilze—eine Art von geduldeter Symbiosis. Flora **116**:331–335. *fig. 1.* 1923.
13. PARKIN, J., On a brilliant pigment appearing after injury in species of *Jacobina* (N.O. Acanthaceae). Ann. Botany **19**:167. 1905.
14. RAVN, K., Nogle Helminthosporium-Arter og de af dem fremkaldte Sygdomme hos Byg og Havre. Bot. Tidssk. **23**:101–321. 1900 (from abstract by STEVENS **17**).
15. REDDY, C. S., and HOLBERT, J. R., The black-bundle disease of corn. Jour. Agric. Res. **27**:177–205. 1924.

16. SMITH, GRANT, The haustoria of the Erysiphaceae. BOT. GAZ. **29**:153–184. *pl. XI–XIII*. 1900.
17. STEVENS, F. L., The *Helminthosporium* foot rot of wheat, with observations on the morphology of *Helminthosporium* and on the occurrence of saltation in the genus. Bull. Ill. State Nat. His. Survey, Art. 5. **14**:77–186. *figs. 17–23*. 1922; also Art. 9. 259–286. 1919 (188 references).
18. WOLF, F. A., The perfect stage of *Actinonema rosae*. BOT. GAZ. **54**:218–234. 1912.
19. WOLFF, R., Der Brand des Getreides. Halle. 1874 (abstract from STEVENS 17).

EXPLANATION OF PLATES XXV–XXVII

Most of the drawings were made with the aid of a projection machine. Magnifications of most of the drawings are shown on the drawings by brackets and measurements in microns. An oil immersion objective was used in making figs. 1, 3, 6, 7, 8, 10, 11, 12, 16, 17, 18, 19, 23, 24, 28, 29, 30, 31, 33, 33*A*. Unless otherwise stated, drawings represent surface views of seedling stems.

PLATE XXV

FIG. 1.—Wheat coleoptile 14 days after inoculation with *Alternaria* from tomato leaf spot: *M*, internal mycelium with penetration hyphae projecting from it and causing callosities in adjacent cell walls; *T*, toothed callosity.

FIG. 2.—Wheat coleoptile 14 days after inoculation with *Alternaria* from apple rot: *P*, penetration hypha 50 μ long; *C*, callosities; swollen host cell walls with prominent middle lamellae shown.

FIG. 3.—Longitudinal section of seedling wheat stem 8 days after inoculation with *Alternaria* from iris: *M*, penetrating cone of mycelium projecting under edge of broken cuticle and causing distinct bending of cell walls below; three cone-shaped masses of mycelium shown in host cells; *H*, host cell wall.

FIG. 4.—Wheat coleoptile 13 days after inoculation with *Alternaria* from gooseberry, showing red stained area bordering penetration hyphae, slightly stained or clear area outside this, and broad, circular band of altered cell wall material deeply stained with Congo red (represented by stippling); cells outside the ring only slightly stained.

FIG. 5.—Wheat coleoptile showing hypha of *Alternaria* from rye causing swellings and some callosities in 7 vertical wheat cell walls which it crosses (7 days after inoculation); appressoria, penetration hyphae, and prominent middle lamellae shown.

FIG. 6.—Two callosities broken out of cells of wheat coleoptile 8 days after inoculation with *Diplodia zeae;* one callosity bears hairlike projection; callosity on left 27 μ long.

FIG. 7.—Appressoria of *Alternaria* from *Datura* 14 days after inoculation on coleoptile of wheat: circles drawn in appressoria represent tops of penetration hyphae; internal mycelium shown in lower cell.

FIG. 8.—Longitudinal section of seedling wheat stem 20 days after inocula-

tion with *Alternaria* from wheat, showing sections of callosities and swollen wheat cell walls; callosities stippled.

FIG. 9.—Wheat coleoptile 9 days after inoculation with *Helminthosporium gramineum*, showing elongate callosities, *E*, containing prominent penetration hyphae, *P*, some of which have free ends.

FIG. 10.—Wheat coleoptile 5 days after inoculation with *Colletotrichum nigrum:* hypha (*H*) bears the appressorium (*A*); peg from center of appressorium penetrated epidermis and was responsible for the altered staining reaction of disk (R), which retained the Congo red stain; middle lamellae prominent in swollen vertical cell walls.

FIG. 11.—Wheat coleoptile stained with Pianeze III B, 8 days after inoculation with *Alternaria* from iris: green region (*G*), contains many callosities (*C*) and two penetration hyphae (*P*); *V*, purple border represented by stippling.

FIG. 12.—Hypha of *Alternaria* from rose in greatly swollen vertical cell wall (*S*) of wheat, 11 days after inoculation: *M*, internal mycelium; swollen cell wall 6 μ in diameter.

FIG. 13.—Wheat coleoptile 14 days after inoculation with *Alternaria* from apple, showing appressorium (*A*), long penetration hypha (*P*), and complex callosity (*C*).

FIG. 14.—Sorghum coleoptile 7 days after inoculation with *Alternaria* from pepper leaf spot, showing characteristic red-brown, auto-stained disk (*D*) surrounding point of incipient penetration.

FIG. 15.—Sorghum coleoptile 7 days after being punctured with point of sterile needle which made the hole (*H*): cells (*B*) were brown: shortest width of hole is 53 μ.

FIG. 16.—Wheat coleoptile 7 days after inoculation with *Alternaria* from corn leaf, showing *Alternaria* spore, germ tube, appressorium, penetration hypha and callosities; penetration hypha is attached to lower side of appressorium; it grew downward, turned, and penetrated vertical cell wall.

FIG. 17.—Epidermis of tomato seedling 6 days after inoculation with *Alternaria* from squash, showing stomatal penetration: *H*, internal hypha which entered through stoma (*S*).

FIG. 18.—Cabbage epidermis 9 days after inoculation with *Alternaria* from *Abutilon*, showing hyphae in greatly swollen vertical cell wall; stippled region was auto-stained yellow.

FIG. 19.—Like fig. 18: end of hypha (*H*) focused in plane slightly above that of vertical cell wall which apparently grew up and inclosed it; *W*, swollen wall.

FIG. 20.—Sorghum coleoptile 7 days after inoculation with *Alternaria* from pepper leaf, showing brown, auto-stained disk under appressorium and peg.

FIG. 21.—Soy bean epidermis 10 days after inoculation with *Alternaria* from bean, showing granular, yellow, auto-stained disk (*Y*); callosity (*C*) contains penetration hypha.

FIG. 22.—Cabbage epidermis 10 days after inoculation with *Alternaria* from tomato rot, showing *Alternaria* spore with germ tube bearing appressorium which causes callosity in vertical cell wall.

FIG. 23.—Surface view of onion bulb membrane 4 days after inoculation with *Alternaria* from onion leaf, showing granular aggregation of irregular particles (*G*) around point of incipient penetration; circle in center of swollen region in cell wall probably represents penetrating hypha; *S*, germinating spore.

PLATE XXVI

FIG. 24.—Muskmelon epidermis 12 days after inoculation with *Alternaria* from iris, showing internal hypha (*I*) in swollen vertical cell wall in which prominent lamellae (*L*) were seen; since the appressoria focus slightly above the internal hypha, the callosities probably grew up and inclosed them.

FIGS. 25, 26.—Muskmelon epidermis 12 days after inoculation with *Alternaria* from iris, showing callosities (*C*), superficial hypha (*H*), and prominent middle lamella (*M*).

FIG. 27.—Pumpkin epidermis 7 days after inoculation with *Alternaria* from iris: *Alternaria* spore (*A*) produced germ tube and appressorium which caused callosity.

FIG. 28.—Soy bean epidermis 10 days after inoculation with *Alternaria* from bean, showing flattened, brown hypha appressed to epidermis and apparently causing yellowing of region around it (*Y*), and swelling of adjacent cell walls.

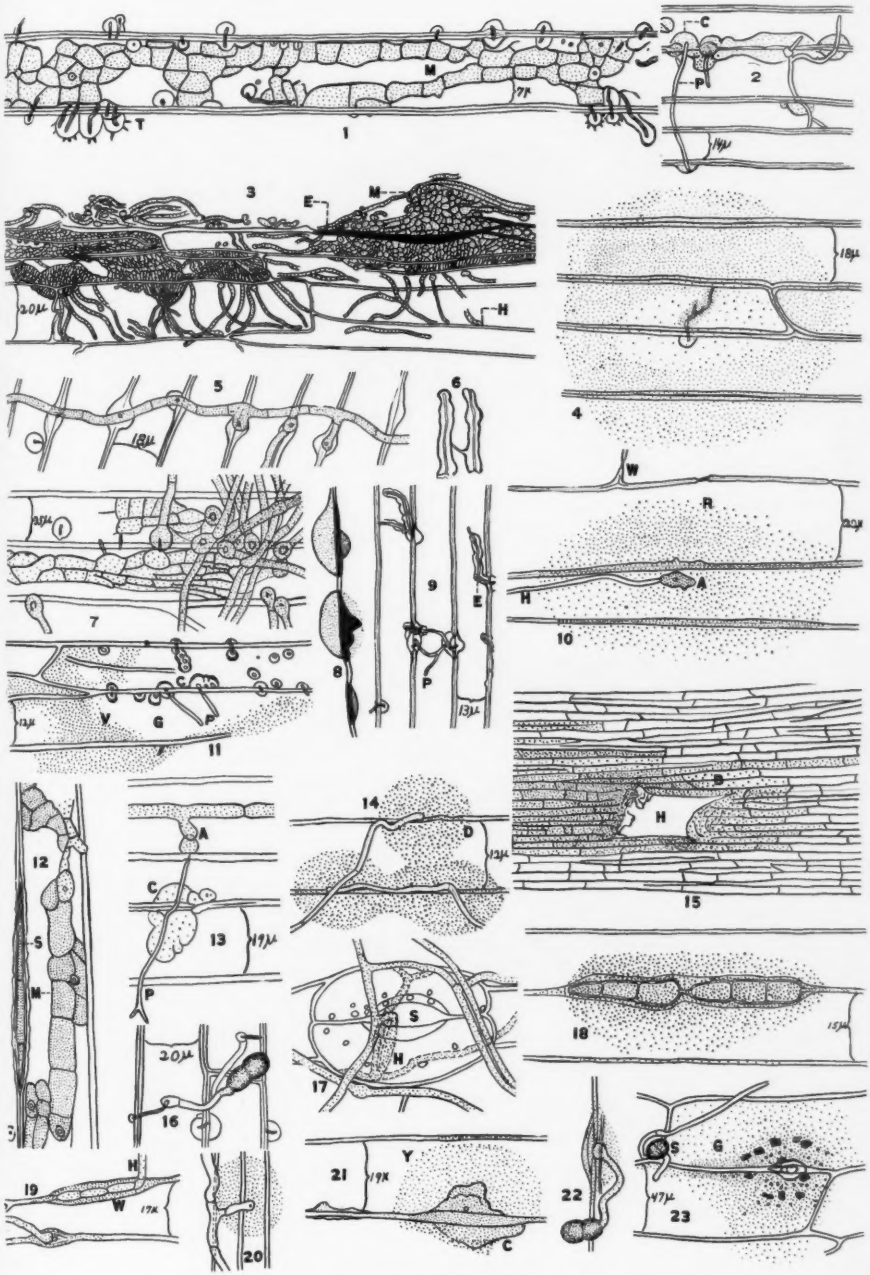
FIG. 29.—Pumpkin hair 7 days after inoculation with *Alternaria* from rose bud mold: hypha (*H*) attached to lower side of hair by branch and appressorium (*A*); callosity, (C_1) in hair focused below plane of plasmolyzed host protoplasm (*P*); C_2, small callosity; middle host cell is 40 μ wide.

FIG. 30.—Soy bean epidermis 10 days after inoculation with *Alternaria* from bean: *C*, callosity; *Y*, yellow, auto-stained, granular region forming indistinct margin of callosity; *G*, granular type of callosity, including swollen host walls which have prominent middle lamellae; *A*, appressorium with penetration hypha causing callosity; *E*, elongate callosity; stippled regions were yellow.

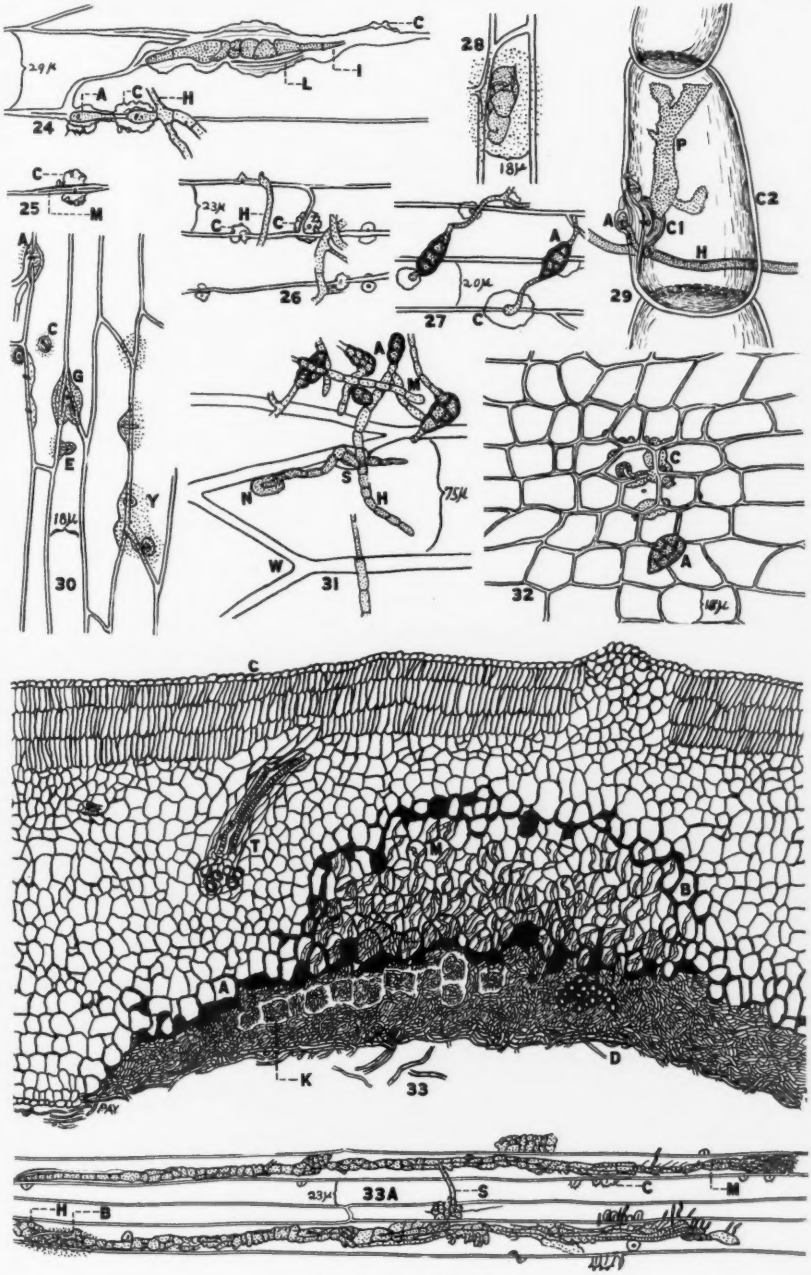
FIG. 31.—Surface view of onion bulb membrane 4 days after inoculation with *Alternaria* from onion leaf spot, showing penetration by hyphae of normal diameter (*H*), which came from spores (*A*) and passed through swollen region in vertical cell wall (*S*); *M*, superficial mycelium; *N*, host nucleus; *W*, host cell wall.

FIG. 32.—Pumpkin epidermis 7 days after inoculation with *Alternaria* from *Abutilon*, showing callosities (*C*) in region of swollen, yellow cell walls which exhibited prominent middle lamellae and contrasted sharply with the thin hyaline cell walls of normal cells surrounding yellow region; *A*, *Alternaria* spore.

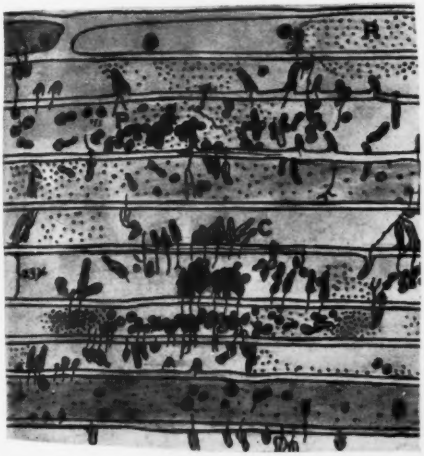
FIG. 33.—Section of pumpkin cotyledon 7 days after inoculation with *Alternaria* from *Abutilon;* dense mass of mycelium (*D*) occupied cavity in lower side of cotyledon and surrounded the small group of tracheae shown above *D;* isolated masses of mycelium (*K*) not yet merged with adjacent mycelial mass (probably because host cell walls had been dissolved away too recently); some sections showed all of outer mycelial mass made up of these blocks of mycelium; *A*, layer of host cells with thickened walls which retained the malachite green



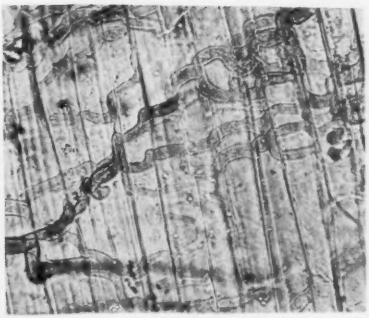
YOUNG on FUNGI



YOUNG on FUNGI

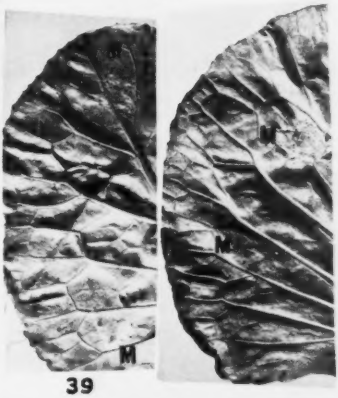


34



36

36A



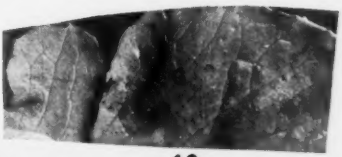
39

35A

35B

38

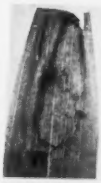
37



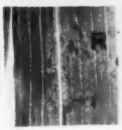
40

42

43



41



YOUNG on FUNGI

stain and form outer boundary; *B*, inner boundary; *M*, scattered mycelium in pocket between boundary layers; *T*, tracheae in region uninvaded by mycelium; *C*, cuticle; slightly diagrammatic.

FIG. 33*A*.—Wheat coleoptile 10 days after inoculation with *Alternaria* from asparagus, showing long ribbons or bands of brown, internal hyphae which sent out penetration hyphae; *M*, brown, internal mycelium; *C*, callosity; *S*, superficial hyphae; *B*, brown region of swollen cell wall which partly surrounds internal hypha; *H*, hypha which is probably internal and focuses above hypha inclosed by wall (*B*); slightly diagrammatic.

PLATE XXVII

FIG. 34.—Wheat coleoptile 7 days after inoculation with *Diplodia zeae:* *P*, penetration hyphae; *C*, yellow, elongate, circular, and complex callosities; *R*, plasmolyzed host protoplasm; *B*, brown cell containing callosities.

FIG. 35*A*.—Photograph of wheat leaves 3 days after inoculation with *Helminthosporium gramineum*, showing green leaf spots in leaves which had turned yellow under bell jar.

FIG. 35*B*.—Reverse of phenomenon shown in fig. 35*A;* common yellow spots in green wheat leaves under same bell jar.

FIG. 36.—Photomicrograph of surface of epidermis of muskmelon seedling stem 8 days after inoculation with *Helminthosporium gramineum*, showing ladder mycelium passing through many host cells, chiefly perpendicular to longer dimensions of cells; some hyphae swollen at points of contact with cell walls and constricted in passing through them.

FIG. 36*A*.—Wheat coleoptile 11 days after inoculation with *Alternaria* from *Datura*, showing radiate and irregular callosities (*H*).

FIG. 37.—Photomicrograph of surface of wheat coleoptile 6 days after inoculation with *Alternaria* from gooseberry, showing long penetration hyphae and callosities.

FIG. 38.—Photomicrograph of surface of wheat coleoptile 7 days after inoculation with *Alternaria* from gooseberry, showing plain, elongate callosities (*E*); toothed elongate callosities (*T*); circular callosities; and brown cell (*B*) containing callosities; other brown cell is 25 μ wide.

FIG. 39.—Cabbage leaves 3 days after inoculation with *Alternaria* from tomato end rot, showing hypophyllous aerial mycelium (*M*) projecting from leaf spots.

FIG. 40.—Green leaf spots caused by *Alternaria* from wheat seeds in radish leaves which had turned yellow under bell jar (4 days after inoculation); ×0.5.

FIG. 41.—Sorghum leaves 3 days after inoculation with *Alternaria* from oats, showing red-brown, auto-stained infection rings (*R*) below rings of spores; ×1.

FIG. 42.—Water-soaked region in radish leaf 3 days after inoculation with *Alternaria* from tomato end rot; ring of spores shown; ×0.5.

FIG. 43.—Green leaf spots caused by *Alternaria brassicae microspora* in radish leaves which turned yellow (*Y*) under bell jar; ×0.5.

DEVELOPMENT OF EMBRYO SAC IN GRINDELIA SQUARROSA

THOMAS D. HOWE

(WITH PLATES XXVIII, XXIX AND ONE FIGURE)

Introduction

GUIGNARD (12) was the first to study the embryo sac of a member of the Astereae. In what he referred to as *Conyza ambigua*, he found that the embryo sac develops in the manner typical of angiosperms, from the lower of a row of four macrospores. After the 8-nucleate stage is reached, three antipodal cells are formed. These persist and increase in number as the embryo sac matures, until there is an antipodal group of about ten cells, each with a single nucleus. SMALL (26) evidently questions the identity of GUIGNARD'S plant, since he refers to it as "*Conyza* sp." HALÁCSY (13) cites *C. ambigua* DC. as synonymous with *Erigeron linifolius* Willd. While GUIGNARD does not give the author of his *C. ambigua*, it is probably DE CANDOLLE'S species, since the only other *C. ambigua* cited in the Index Kewensis is a native of South America.

NORRIS (19) found that in *Grindelia squarrosa* (Pursh) Dunal, a typical embryo sac develops from the chalazal macrospore. In the mature embryo sac there are usually two antipodal cells; in rare cases there are three. The lower cell develops a "vermiform extension," which grows through the "endodermis," and which he thought aids in the breaking down of the cells of the integument. It was not figured as extending beyond the first layer of these cells.

MARTIN (18) gave the first account of the embryo sac of *Aster* and *Solidago*. He does not specify from which genus his figures were drawn. The embryo sac develops to the 8-nucleate stage in the typical manner. There are usually three antipodal nuclei, although occasionally four were observed. In the former case, these nuclei are arranged in a triangle with no walls between them. Later stages were not observed.

CHAMBERLAIN (6) studied *Aster novae-angliae*, observing especially the antipodal cells. Contrary to the observations of MARTIN,

he found that the three antipodal nuclei are arranged in a linear row and are separated by cell walls. The antipodal cells increase in number, forming a row of cells, sometimes as many as twenty. This antipodal haustorium extends into the basal part of the ovule. What was thought to be an "antipodal oosphere" was observed in one instance in the basal antipodal cell. This account was confirmed by Miss GOLDFLUSS (11) and Miss OPPERMAN (20). The latter also reported the presence of an antipodal haustorium in *A. undulatus* as well as in *A. multiflorus*. An "antipodal oosphere" with a male nucleus about to unite with it was found in one case in *A. undulatus*. LAND (17), in a study designed primarily to determine the question of the occurrence of double fertilization, reported a varying number of antipodal cells in *Erigeron philadelphicus* and in *E. strigosus*. It seems to have become generally accepted that in the Astereae the antipodal cells persist and develop a chalazal haustorium (COULTER and CHAMBERLAIN 8).

A new interpretation was given by PALM (21) to this haustorial structure. In *Aster novae-angliae* he concluded that the haustorium is formed from persistent macrospores when one of the spores other than the chalazal one develops into the embryo sac. In *Solidago serotina* the micropylar spore is always the functional one, and the others form a haustorium. As evidence, he figures rows of three or four cells with two or four nuclei in the micropylar cell, and later stages showing five or six nuclei in the micropylar cell and one or two in each of the others. In *A. sibericus* the chalazal macrospore always functions. In a later paper (22) he reported that in *A. novi-belgii* the chalazal spore always functions, and that the antipodal cells do not develop a haustorium. In *Bellis perennis* the chalazal macrospore usually develops into the embryo sac. Rarely one of the others functions and the chalazal spore enlarges, forming a cell which CARANO (3) had interpreted as being of antipodal origin. In a publication dealing with the different types of angiosperm embryo sacs (23), PALM gave more facts substantiating his interpretation of this large cell in *Bellis perennis*. In *Emilia* (*Senecio*) *sagittata* he found that any one of the macrospores may function. When the chalazal spore develops into an embryo sac the antipodal cells persist. When one of the other spores functions, the deeper macrospores persist

and enlarge. The cytoplasm of the antipodal cells is denser and less vacuolate than is that of the macrospores. According to AFZELIUS (1), PALM's plant is really *Emilia amplexicaulis*. CHAMBERLAIN (7) pointed out that the figures given by PALM to support his position are not entirely convincing, and reasserted his opinion that the haustorium in *Aster* and *Solidago* is of antipodal origin.

CARANO (5) studied *Aster novae-angliae* and three species of *Solidago*. In *Aster* any one of the macrospores may develop into an embryo sac, although it is more often the chalazal one which thus functions. The disintegration of the non-functional macrospores was observed in nearly all cases. A few examples were found in later stages of the apparent persistence of a macrospore other than the functional one, but such a persistent macrospore never enlarges to form the haustorium, which latter is always derived from the antipodal cells, at first three in number. CARANO decided that the "antipodal oosphere" is really an enlarged cell of the integument, since in one instance he observed the diploid chromosome number in such a cell.

In *Solidago serotina* and *S. canadensis*, according to CARANO, the chalazal macrospore always develops into the embryo sac. In these species there are at first two or three antipodal cells, although there may be four in later stages. A plant which the writer calls *S. Ridellii* is similar to the others so far as observed. Doubt is expressed as to its identity. In *Bellis perennis* the large cell in the chalazal part of the embryo sac is really derived from an antipodal cell, and not from a persistent macrospore.

In *Erigeron glabellus*, CARANO found many archesporial cells in the nucellus. Each archesporial cell divides, but the second nuclear division is usually not followed by cell division. The lower of the two binucleate cells derived from each archesporial cell develops into an embryo sac, thus following the *Scilla* type of development. The mature sac usually contains two antipodal cells, but they may be suppressed in consequence of competition with neighboring embryo sacs. *Erigeron Karwinskianus* var. *mucronatus* is usually parthenogenetic, and the eight nuclei of the embryo sac are formed by three equatorial nuclear divisions. The number of nuclear divisions is evidently variable, since in one sac ten spindles were observed.

DAHLGREN (9) observed that in *Erigeron aurantiacus* a typical embryo sac is developed. In *Aster capensis* a chalazal haustorium was found, but its origin was not determined. *Chrysocoma coma-aurea* has three antipodal cells, which may be arranged either in a triangle or in a row.

HOLMGREN (15), following a note by TAHARA (28) reporting the occurrence of parthenogenesis in *Erigeron annuus*, studied several species of *Erigeron*. The embryo sac of *E. bonariensis* develops in the typical manner. *E. unalaschkensis* and *E. Coulteri* follow the *Scilla* type of development. In *E. eriocephalus* and *E. politus*, cell division does not occur during meiosis, and a 16-nucleate embryo sac is formed after two more nuclear divisions. The material which he referred to *Erigeron annuus* was somewhat different from the typical form, and cannot be identified with certainty because of the well known occurrence of polymorphism within this genus. The plant is parthenogenetic, and the nuclei of the embryo sac are formed by three equational nuclear divisions in the archesporial cell. The two antipodal cells remain small and later degenerate.

TAHARA (28) published a more complete account of *Erigeron annuus* in connection with the results of studies on *E. linifolius* and *E. dubius*. *E. annuus* is parthenogenetic, the embryo sac nuclei being formed by three equational divisions. The mature embryo sac usually contains only seven nuclei, in consequence of the failure of the chalazal nucleus at the 4-nucleate stage to divide. The two antipodal cells degenerate as the embryo develops. In *E. linifolius* the embryo sac is formed in the typical manner from the chalazal macrospore. After the 8-nucleate stage, two antipodal cells are formed which persist and divide. The two nuclei of the micropylar antipodal cell often fuse before the cell divides, and, since there are nuclear fusions in later formed cells, high chromosome numbers are found at later stages. In *E. dubius* cell walls are not formed between the macrospore nuclei. Usually each of the daughter nuclei formed by their division divides again, the final result being a 16-nucleate embryo sac with eleven antipodal cells arranged in a compact tissue. If some of the nuclei fail to divide, fewer antipodal cells are formed.

PALM (24) found that in *Vittadinia trilobata* the macrospore nuclei are not separated by cell walls, although there is a vacuole

between each two adjacent nuclei. The two chalazal nuclei are slightly smaller than the others, and gradually disintegrate, while the two micropylar nuclei form the embryo sac by means of two successive nuclear divisions. After the second nuclear division, cell plates are formed on the spindles, dividing the sac into six cells. The two antipodal cells persist and increase in number, until there are ten or twelve arranged in a single row.

In 1922 the writer (**16**) published a preliminary note concerning the embryo sac of *Grindelia squarrosa*. At that time the evidence indicated that the micropylar macrospore develops the embryo sac, and that at least two of the other macrospores persist. It was thought that the non-functional macrospore nearest the embryo sac grows out into the integument, while the other non-functional macrospore enlarges without forming an outgrowth. Later studies have shown that the two cells which thus increase in size are really antipodal cells, and that it is the chalazal macrospore which forms the embryo sac.

Materials and methods

The material used in this investigation was collected near Lincoln, Nebraska, over the period of years from 1920 to 1923. The heads were cut into slices before being placed in the fixing solution. A number of fixatives were tried, and none was entirely satisfactory. A saturated aqueous solution of mercuric chloride with 5 per cent acetic acid gave the least plasmolysis in the developing embryo sac. It obscured the spindle fibers, and after its use staining with safranin was difficult. Flemming's stronger solution gave fair results for the developing macrospores, but it had the disadvantage of affecting the oil droplets so that they took the safranin stain. It was unsatisfactory for later stages. Zenker's solution also gave fair results. Chloroform, xylol, and cedar oil were used to precede infiltration with paraffin. Great difficulty was encountered in cutting the sections because of the hardness of the material. Cutting was more satisfactory after the use of cedar oil. Sections were cut from 5–10 μ thick. Flemming's triple stain and Haidenhain's iron-alum haematoxylin were used. Orange G was sometimes used as a counterstain with the haematoxylin, in order to make the cell walls visible. In the earlier part of the work, gold orange was used instead of orange G because it acted more rapidly.

Macrospore development

The ovule of *Grindelia squarrosa* is similar to the ovules of most other Sympetalae that have been investigated, in having one thick integument. This integument is not distinct on the funicular side of the ovule from the funiculus itself. In the earliest stages studied the integument had nearly grown around the nucellus, which contains one cell larger than its neighbors and with a larger nucleus (text fig. 1*A*). Observations of later stages show that this large cell is the archesporial cell, which functions directly as the macrospore mother cell. The nucellus of this ovule is shown at a greater magnification in fig. 1. No ovules were observed that contained more than one archesporial cell. The nucleus usually passes into synizesis before the integument is fully developed. At this stage there is a typical aggregation of the chromatin threads at one side of the nucleus (fig. 2). In the ovules of one head the nuclei of the archesporial cells were still in the resting condition, although the integuments were fully developed (text fig. 1*B*). One example of the heterotypic equatorial plate was observed (fig. 3). The chromosomes are small, and their number could not be determined with certainty. Since the diploid number is twelve, as will appear later, there are probably six bivalent chromosomes at this time, although only four are distinctly visible on the equatorial plate figured. A cell wall is formed after the heterotypic division, as is usually the case in Compositae (SMALL **26**). The homoetypic mitosis was not observed. That it occurs, however, is shown by the fact that four substantially equal cells are formed which lie in a row (fig. 4). The macrospore tetrad is thus of the type most frequently observed in angiosperms.

Embryo sac development

The chalazal macrospore always develops into the embryo sac. In fig. 6 it is shown in process of enlargement; the other macrospores are disintegrating, as is shown by the homogeneous cytoplasm and by the fact that their nuclei have become dark-staining masses. The cells of the nucellus adjacent to the developing spore are beginning to degenerate. In the section shown in fig. 5, both the chalazal spore and the second spore from the micropyle are enlarging. Fig. 9 shows a later stage in the development of such an ovule, and it is evident in this case that the chalazal spore will develop into an em-

bryo sac. The protoplasm of two of the other spores has lost its structure; and while the nucleus of the second spore from the micropyle is still visible, its reticulum is not seen and its cytoplasm has become coarsely granular. No instances were found in which the chalazal spore was disintegrating. The non-functional macrospores gradually disappear, and all trace of them is lost while the functional one is still inclosed by the nucellus, the cells of which have become structureless masses (fig. 8). The macrospore then, continuing to grow, breaks through the micropylar end of the nucellus, and its later development takes place in contact with the cells of the integument. The remnants of the nucellar cells remain visible about the chalazal portion of the embryo sac until a much later period (fig. 29).

While these changes are taking place, the cells of the inner layer of the integument elongate radially. This elongation is first apparent in the macrospore tetrad stage (fig. 4). The cytoplasm of these cells becomes dense, and they form the "epithelial" layer characteristic of the Compositae. Longitudinal divisions evidently occur, since at later stages the cells of the layer in question are much narrower (fig. 30). Two nuclei were sometimes found in one of these cells, but no evidence of amitosis was observed. The tissue next outside this epithelial layer gradually disintegrates as the ovule matures (fig. 28).

The nucleus of the functional macrospore does not divide until this spore has grown through the micropylar end of the nucellus. Fig. 9 shows a binucleate embryo sac just after nuclear division, the stage being determined by the presence of a spindle. A vacuole appears at each end of the embryo sac, which latter continues to enlarge. One of the daughter nuclei then moves toward the micropylar end of the embryo sac, the vacuole in that part of the sac thus coming to be between the two nuclei (fig. 10). In the section shown in fig. 10, the vacuole has collapsed during fixation. Each nucleus divides (fig. 11), and the daughter nuclei of this division move apart (fig. 12). A vacuole appears between the two chalazal nuclei after this division. Figs. 13 and 14 show each an early 8-nucleate stage, with one spindle still visible in fig. 13 and two in fig. 14. Fig. 14 shows the usual relative position of the egg nucleus and the upper polar nucleus with a cell membrane between them. The embryo sac when fully mature contains six cells, the upper of the two antipodal cells containing

two nuclei (fig. 15). In the section represented in this figure the wall of the embryo sac (macrospore wall) is visible at one point where it passes over the end of the degenerating nucellar cells. This wall also appears in the section shown in fig. 31. Cell division between the two antipodal cells and between the micropylar antipodal cell and the primary endosperm cell occurs by means of the formation of cell plates in the corresponding spindles. In the section shown in fig. 13, the phragmoplast extends to the plasma membrane of the embryo sac. Fig. 18 is an enlarged representation of the lower polar nucleus and of what is to be the micropylar nucleus of the upper antipodal cell of the embryo sac shown in fig. 14. The phragmoplast extends to the plasma membrane of the cell, and the completed portion of the cell plate has split. The halves of the cell plate have become separated during fixation. The method of cell division resulting in the formation of the egg and the synergids was not determined. From the position of the two micropylar nuclei shown in figs. 13 and 14, it is evident that they are to be the nuclei of the synergids appearing in fig. 15. Cell walls are formed between the two antipodal cells and between the micropylar antipodal cell and the primary endosperm cell. These walls are not apparent in fig. 15; but since they occur at all later stages (for example, fig. 29), it is probable that they had not yet been formed in the embryo sac shown in the former figure. Definite walls in this position have been figured by previous writers for several composites (DAHLGREN **10**, TÄCKHOLM **27**).

The polar nuclei fuse at the micropylar end of the primary endosperm cell in contact with the membrane separating this cell from the egg (fig. 19), their union forming a nucleus that is much larger than the egg nucleus (fig. 20). The synergids extend into the micropyle; each contains a vacuole in the end nearer the egg. The egg is somewhat pear-shaped, with its broader end away from the micropyle. In the drawings the micropylar end of the egg is hidden by the synergids. The egg apparatus resembles that figured by LAND (**17**) for *Erigeron philadelphicus*.

Fertilization

Although the remains of the pollen tube were seen in several embryo sacs (for example, fig. 25), only one case of fertilization was observed (fig. 21). One synergid is destroyed by the pollen tube as it

enters. There seems to be no marked difference in size or shape between the egg nucleus and the male nucleus at the time of their union (fig. 22). According to LAND, this is also the case in *Erigeron philadelphicus*. The primary endosperm nucleus of the same embryo sac (fig. 23) shows a protuberance which quite probably represents the second male nucleus, but this fact could not be determined with certainty. The primary endosperm nucleus divides before the zygote nucleus (fig. 24), and in consequence of repeated nuclear divisions free endosperm nuclei are formed. Cell division in the endosperm does not begin until after the beginning of the development of the embryo (fig. 25). In *Aster* and *Solidago* (CARANO **5**) the first division of the primary endosperm nucleus is followed by cell wall formation, and there is no stage at which free nuclei appear in the endosperm. In some other Compositae, however, such as *Helianthus annuus* and *Centaurea scabiosa* (DAHLGREN **10**), free nuclei are found after the embryo has begun to develop. According to the usual classification, *Grindelia* is not closely related to those members of the Astereae which have been investigated, so that a different method of endosperm development is not surprising.

Antipodal cells

The nucleus of the chalazal antipodal cell almost always divides. In most cases the cell enlarges and grows outward into the integument. In fig. 29 it is shown growing through the epithelial layer, a stage similar to that figured by NORRIS (**19**). It often grows into the integument for a considerable distance, forming a lateral haustorium (fig. 31). No instances were found in which it had grown directly toward the base of the ovule to form the more frequently described type of chalazal haustorium. The antipodal cell nearer the micropyle almost always persists, and usually also grows into the integument to form a lateral haustorium. This development takes place at about the time the embryo sac is mature. In fig. 30, showing a part of the same embryo sac represented in fig. 20, this second antipodal cell has just grown through the epithelial layer of the integument. The lateral haustorium or haustoria thus developed extend out into the tissue of the integument for a considerable dis-

tance. The one nearer the micropyle often grows through the degenerating cells surrounding the epithelial layer, into the more solid tissue of the ovule (fig. 27), the degenerating cells being shown in the figure by broken lines. In fig. 26 such a haustorium appears within two cell layers of the surface of the ovule. The direction of growth of this haustorium is usually not strictly perpendicular to the long axis of the embryo sac, but is inclined somewhat toward the micropylar end of the ovule. Fig. 31 shows lateral haustoria from both antipodal cells appearing in the same section. These haustoria may grow into that part of the ovule in which the integument is not distinct from the funiculus, as well as into the integument itself. In practically all mature embryo sacs seen, at least one such haustorium was observed. Several nuclear divisions occur in these proliferating antipodal cells, but cell division was never observed. The cytoplasm of a haustorium is densest near its distal end, and it is in this dense cytoplasm that most of the nuclei are found. In fig. 28 five nuclei are shown near the end of a lateral haustorium derived from the micropylar antipodal cell. Two nuclei are much larger than the other three; and since each has two nucleoli, it might be supposed that they had been formed by the fusion of nuclei. The three smaller nuclei are in close contact, as though they might be in process of fusion. Nuclear fusions in antipodal cells were reported by TAHARA (**29**) for *Erigeron linifolius*. The cells of the integument adjacent to a haustorium show no more evidences of degeneration than do other cells equally distant from the epithelial layer and not in contact with a haustorium. There is thus no evidence that the haustoria serve as active agents in the disintegration of the cells of the integument. The antipodal outgrowths persist until they are absorbed by the developing embryo.

NORRIS (**19**) mentions that in rare instances three antipodal cells were found in *Grindelia squarrosa*. No such cases were observed in the material that I have studied.

Several-ovuled ovaries

In two heads of flowers nearly every ovary contained either two or three cavities with an ovule in each. In the section shown in

text fig. 1*C* only one of the ovules appears, the other being in another section. In most of the ovules in these heads the development of the

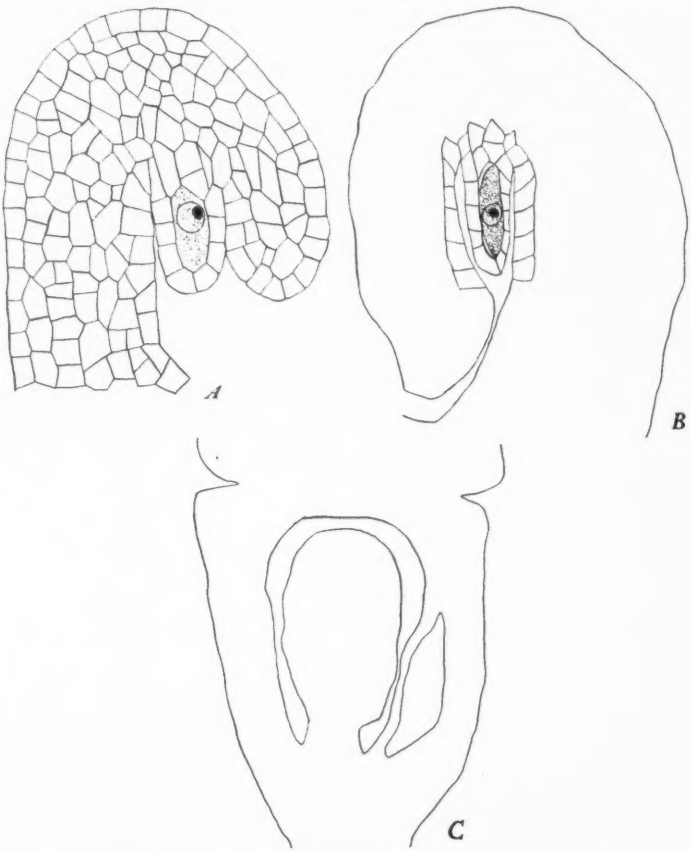


FIG. 1.—*A*, young ovule, showing integument nearly grown around nucellus, which contains archesporial cell, ×330; *B*, unusual ovule, in which nucleus of macrospore mother cell is still in resting condition after integument has grown around nucellus, ×330; *C*, unusual ovary containing two cavities, showing ovule in one; another section shows ovule in the other cavity; ×85.

chalazal macrospore into an embryo sac was in progress. No stages were found showing earlier or later stages of such ovaries. AFZELIUS

(1) found that occasionally the ovary of *Senecio disciflorus* has two cavities, each with an ovule. Miss E. N. ANDERSON[1] found that in various Compositae the ovary has three cavities when the style has three branches, but only two cavities in any case contain ovules. In the present material the number of stylar branches was not determined, since the unusual condition of the ovaries was not noticed until after the sections had been cut.

Chromosome number

Chromosome counts made from mitotic figures in the developing ovules showed twelve to be the diploid number (fig. 16). Division of the nuclei in the embryo sac seems to be nearly simultaneous, since three of the four nuclei were in process of division in the only sac in which mitosis was observed (fig. 17). These figures, while not conclusive in themselves, agree with the expectation that six chromosomes (the haploid number) would be present on the gametophytic spindles. The only mitotic figures seen in the endosperm were of the first division in each of two ovules (fig. 24). These figures indicate that more than twelve chromosomes were present, although the number could not be determined with certainty. These last mentioned divisions thus furnish additional evidence that typical double fertilization occurs.

Discussion

The development of the embryo sac of *Grindelia squarrosa* to the 8-nucleate stage is essentially like that of most other Compositae that have been studied. The brief description of NORRIS (19), so far as it goes, agrees with the account given by the writer. The extensive development of lateral haustoria from the antipodal cells, however, is apparently unlike anything previously reported for any plant, although NORRIS gives one figure of what is probably an early stage in the development of such a structure. Aside from this one doubtful reference, two types of haustoria have been described in the Compositae: those which are derived from antipodal cells and grow directly toward the base of the ovule, and those which are derived from synergids and grow into the tissue surrounding the micropyle

[1] Unpublished thesis, University of Nebraska.

of the ovule. A chalazal haustorium formed by the aggressive development of the antipodal cells has been known since GUIGNARD'S study of *Conyza ambigua*. This structure is especially well developed in *Aster novae-angliae*, where CARANÓ (5) has definitely shown that it is of antipodal origin. A summary of the number and character of the antipodal cells in Compositae which have been investigated is given by SMALL (26).

A micropylar outgrowth in *Calendula arvensis* was first figured by TULASNE (30), who considered it to be an extension of the suspensor of the embryo. HOFMEISTER (14) gave evidence to show that it is really an enlarged synergid. BILLINGS (2) found that in seven species of *Calendula* a similar haustorium occurs, and traced its development from a synergid. This account was confirmed by CARANÓ (4). DAHLGREN (10) found that in *Ursinia anthemoides* the synergids elongate and may form a haustorium which grows into the funiculus at the base of the ovule. Haustoria of this nature are generally supposed to have a nutritive function, breaking down the tissue of the integument and absorbing food substances for the developing embryo. No evidence that they serve as active agents in disintegrating the cells was found in *Grindelia squarrosa*.

SHARP (25) has described a lateral haustorium in *Physostegia*, formed by the endosperm. In this case, however, the haustorium grows toward the base of the ovule and forces the antipodal cells into a lateral position. At least one of the antipodal cells persists, but it does not increase in size.

Grindelia also differs from other members of the Astereae hitherto investigated in that there are at first free nuclei in the endosperm. As already pointed out, this plant is not considered to be closely related to any one of those previously studied.

Summary

1. The ovule of *Grindelia squarrosa* has one thick integument; the nucellus consists of one layer of cells outside the archesporial cell, which latter functions directly as a macrospore mother cell.

2. The macrospore tetrad consists of a row of four substantially equal cells, of which the chalazal one always gives rise to the embryo sac.

3. After the 8-nucleate stage is reached the sac is divided into six cells, the upper (micropylar) antipodal cell having two nuclei. Cell division between the two antipodal cells and between the micropylar antipodal cell and the primary endosperm cell takes place by means of cell plates on the corresponding spindles. Cell walls are later formed between these cells.

4. The two antipodal cells persist but do not divide. One or both of them grow laterally into the integument, thus forming one or two lateral haustoria, which may extend nearly to the surface of the ovule.

5. Typical double fertilization probably occurs. The fusion of the egg nucleus with one male nucleus was observed.

6. The primary endosperm nucleus divides before the zygote nucleus. Several free endosperm nuclei are formed before cell division occurs within the endosperm.

7. Twelve is the diploid chromosome number.

I wish to express my appreciation and thanks to Dr. E. R. WALKER of the University of Nebraska, under whose direction this work was begun, and to Dr. C. E. ALLEN, under whose direction it was completed, for helpful advice and suggestions.

JAMES MILLIKIN UNIVERSITY
DECATUR, ILL.

LITERATURE CITED

1. AFZELIUS, K., Embryologische und zytologische Studien in *Senecio* und verwandten Gattungen. Acta Hort. Berg. **8**:123–219. 1924.
2. BILLINGS, F. H., Beiträge zur Kenntniss der Samenentwicklung. Flora **88**: 253–318. 1901.
3. CARANO, E., Su particolari anomalie del sacco embrionale di *Bellis perennis*. Annali Bot. **11**:435–439. 1913 (cited by PALM **21**).
4. ———, Ricerche sull' embryogenesi delle Asteraceae. Annali Bot. **13**:251–301. 1915.
5. ———, Nuove ricerche sulla embriologie delle Asteraceae. Annali Bot. **15**: 97–196. 1921.
6. CHAMBERLAIN, C. J., The embryo sac of *Aster novae-angliae*. BOT. GAZ. **20**:205–212. 1895.
7. ———, The embryo sac of *Aster* and *Solidago*. BOT. GAZ. **65**:571–572. 1918.

8. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of angiosperms. 1903.
9. DAHLGREN, K. V., Zur Embryologie der Kompositen mit besonderer Berücksichtigung der Endospermbildung. Zeitschr. Bot. **12**:481–516. 1920.
10. ———, Studien über die Endospermbildung der Kompositen. Svensk Bot. Tid. **18**:177–203. 1924.
11. GOLDFLUSS, MATHILDE, Sur la structure et les fonctions de l'assise epithéliale et des antipodes chez les Composées. Jour. Botanique **12**:374–384. 1898. *Ibid.* **13**:9–17; 49–59; 87–96. 1899.
12. GUIGNARD, L., Recherches sur le sac embryonnaire des phanerogames angiospermes. Ann. Sci. Nat. Bot. VI. **13**:136–199. 1882.
13. HALÁCSY, E. DE, Conspectus florae Graecae. Vol. II.
14. HOFMEISTER, W., Neuere Beobachtungen über Embryobildung der Phanerogamen. Jahrb. Wiss. Bot. **1**:82–188. 1858.
15. HOLMGREN, I., Zytologische Studien über die Fortpflanzung bei den Gattungen *Erigeron* und *Eupatorium*. Kungl. Svensk. Vet.-Akad. Handl. **59**^{7}:^{1} 1919.
16. HOWE, T. D., A preliminary notice concerning the embryo sac of *Grindelia squarrosa*. Publ. Nebr. Acad. Sci. **10**^{3}:116. 1922.
17. LAND, W. J. G., Double fertilization in Compositae. BOT. GAZ. **30**:250–260. 1900.
18. MARTIN, G. W., Development of the flower and embryo sac in *Aster* and *Solidago*. BOT. GAZ. **17**:353–358; 406–411. 1892.
19. NORRIS, W. W., Development of the ovule in *Grindelia squarrosa*. Amer. Nat. **26**:703–705. 1892.
20. OPPERMAN, MARIE, A contribution to the life history of *Aster*. BOT. GAZ. **37**:353–362. 1904.
21. PALM, B., Zur Embryologie der Gattungen *Aster* und *Solidago*. Acta Hort. Berg. **5**^{4}: 1914.
22. ———, Über die Embryosackentwicklung einiger Kompositen. Svensk Bot. Tidskr. **8**:447–453. 1915.
23. ———, Studien über Konstructionstypen und Entwicklungswege des Embryosackes der Angiospermen. Inaug. Diss. Stockholm. 1915.
24. ———, B., The embryo sac of *Vittadinia*. Ann. Jard. Bot. Buitenzorg **32**: 88–95. 1922.
25. SHARP, L. W., The embryo sac of *Physostegia*. BOT. GAZ. **52**:218–225. 1911.
26. SMALL, J., The origin and development of the Compositae. XI. Miscellaneous topics. New Phytol. **18**:129–276. 1919.
27. TÄCKHOLM, G., Zur Antipodenentwicklung der Kompositengattungen *Cosmidium* und *Cosmos*. Svensk Bot. Tidskr. **10**:423–437. 1916.
28. TAHARA, M., Parthenogenesis in *Erigeron annuus* (a preliminary note). Bot. Mag. Tokyo **29**:245–254. 1915 (cited by HOLMGREN **14**).
29. ———, Cytologische Studien an einigen Kompositen. Jour. Coll. Sci. Tokyo Imper. Univ. **43**^{7}: 1921.

30. TULASNE, L. R., Nouvelles études d'embryogénie vegétale. Ann. Sci. Nat. IV. 4:65-122. 1855.

EXPLANATION OF PLATES XXVIII, XXIX

All drawings were made with the aid of a camera lucida. The actual magnification allowing for the reduction is given in each case.

PLATE XXVIII

FIG. 1.—Nucellus, showing archesporial cell (macrospore mother cell); ×820.

FIG. 2.—Synizesis in macrospore mother cell; ×2100.

FIG. 3.—Heterotypic equatorial plate in macrospore mother cell; ×2100.

FIG. 4.—Macrospore tetrad, with surrounding cells of integument showing beginning of elongation; ×820.

FIG. 5.—Macrospores; both chalazal spore and second one from micropyle beginning to enlarge; reconstructed from two sections; ×820.

FIG. 6.—Macrospores, showing chalazal one enlarging and others in process of degeneration; reconstructed from two sections; ×820.

FIG. 7.—Macrospores, chalazal one enlarging and others in process of degeneration; nucleus of second spore from micropyle still visible; ×820.

FIG. 8.—Functional macrospore, after others have entirely disappeared; reconstructed from two sections; ×820.

FIG. 9.—Binucleate embryo sac just after first nuclear division; macrospore has grown through end of nucellus and is in contact with integument; ×820.

FIG. 10.—Binucleate embryo sac after one of daughter nuclei has moved to near micropylar end of sac; remnants of nucellar cells visible at chalazal end of sac; reconstructed from two sections; ×500.

FIG. 11.—Two ends of 4-nucleate embryo sac just after nuclear division; drawn from two sections; ×500.

FIG. 12.—Four-nucleate embryo sac after daughter nuclei have moved apart; ×500.

FIG. 13.—Eight-nucleate embryo sac; remnants of nucellar cells still visible; phragmoplast of spindle of third nuclear division has reached plasma membrane of embryo sac; ×500.

FIG. 14.—Eight-nucleate embryo sac, showing more usual relative position of egg nucleus and upper polar nucleus; phragmoplast of spindle of last division has reached plasma membrane of embryo sac, and cell plate has split; ×500.

FIG. 15.—Embryo sac after all cells have been formed, surrounded by epithelial layer; ×380.

FIG. 16.—Equatorial plate from developing ovule, showing twelve chromosomes; ×2500.

FIG. 17.—Equatorial plate from embryo sac, showing six chromosomes; ×2500.

FIG. 18.—Enlarged representation of lower polar nucleus and one antipodal nucleus shown in fig. 14 showing phragmoplast and split cell plate; ×2500.

FIG. 19.—Micropylar end of embryo sac, showing fusion of polar nuclei; primary endosperm cell separated from upper antipodal cell by cell wall; ×820.

FIG. 20.—Micropylar end of embryo sac after fusion of polar nuclei; ×820.

PLATE XXIX

FIG. 21.—Fertilization, showing fusion of egg and male nuclei, and empty pollen tube: ×1000.

FIG. 22.—Egg and male nuclei fusing; ×2100.

FIG. 23.—Primary endosperm nucleus of same embryo sac of which the egg is shown in fig. 22; protuberance probably represents second male nucleus, ×2100.

FIG. 24.—First division of primary endosperm nucleus; zygote nucleus has two nucleoli; ×2100.

FIG. 25.—First division of zygote nucleus; free endosperm nuclei and remains of pollen tube shown; ×850.

FIG. 26.—End of lateral haustorium from micropylar antipodal cell which has extended to within two layers of cells of surface of ovule; ×500.

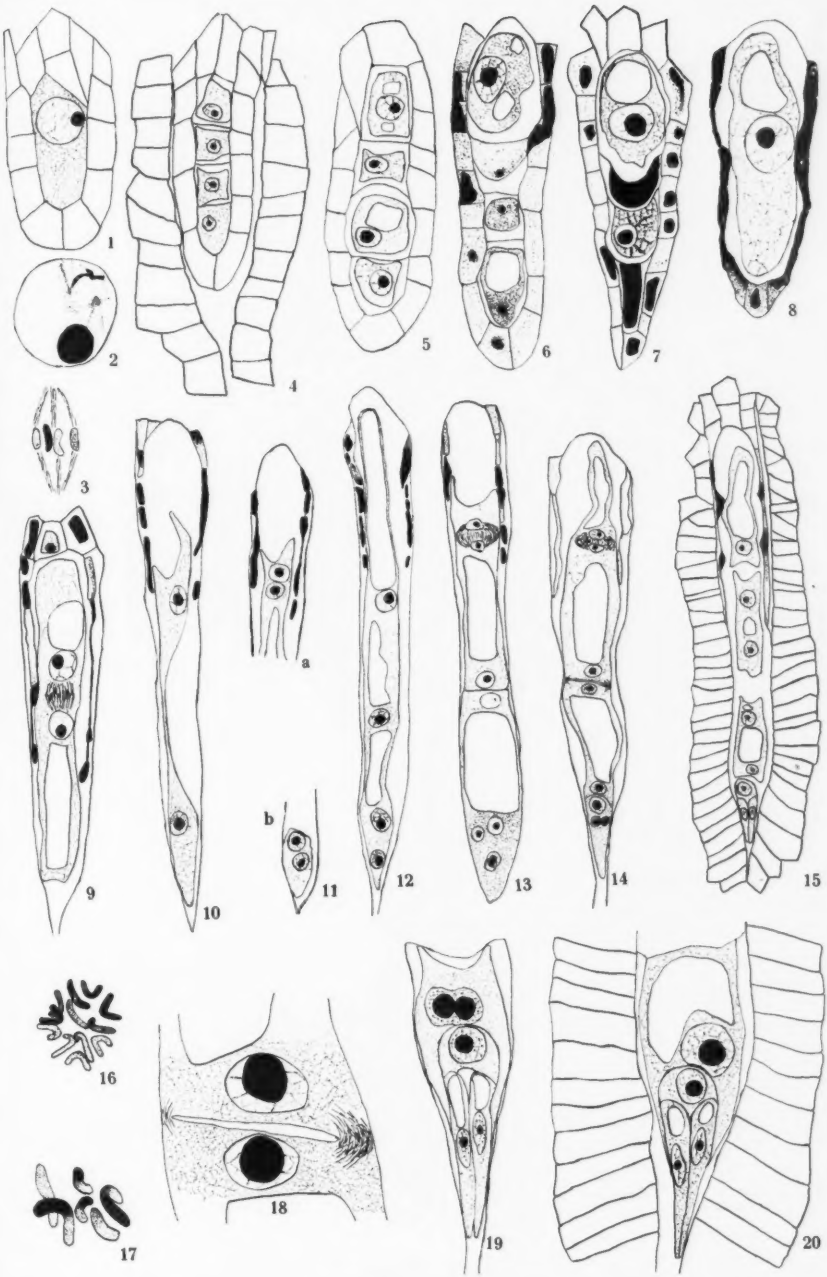
FIG. 27.—End of lateral haustorium from micropylar antipodal cell which has reached the more solid tissue of ovule; three nuclei shown; ×500.

FIG. 28.—Lateral haustorium from micropylar antipodal cell containing five nuclei, two being much larger than other three; ×500.

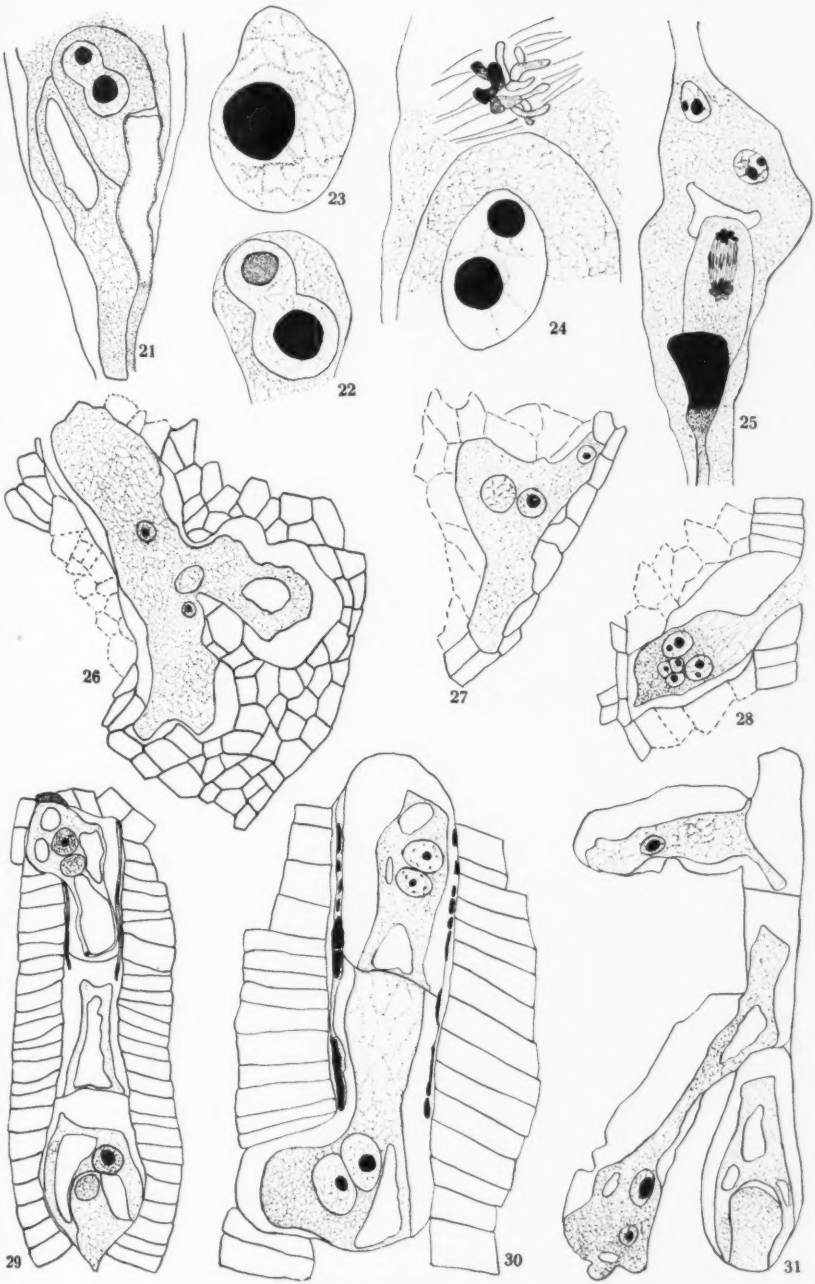
FIG. 29.—Embryo sac in which chalazal antipodal cell has grown through epithelial layer; ×500.

FIG. 30.—Antipodal cells of embryo sac shown in fig. 20; micropylar antipodal cell grown through epithelial layer; remnants of nucellus still visible; ×820.

FIG. 31.—Embryo sac, showing lateral haustoria from both antipodal cells; ×380.



HOWE on GRINDELIA



HOWE on GRINDELIA

EFFECT OF THIOUREA UPON BUD INHIBITION AND APICAL DOMINANCE OF POTATO[1]

F. E. DENNY

(WITH SEVEN FIGURES)

Introduction

At each "eye" of the potato (*Solanum tuberosum*) are the rudiments of several buds (three or more, according to ARTSCHWAGER 3). Usually only one of these buds will develop, the growth of the accessory buds being inhibited. If the sprout which first starts is removed, however, then one of the other buds will begin active growth. Whatever may be the fundamental cause of the repression of these supernumerary buds by the dominant bud, it is shown in this paper that treating the cut tuber with a solution of thiourea (NH_2CSNH_2) causes the growth of two or more (often four or five) buds from a single eye.

The tuber of *Solanum tuberosum* is morphologically a thickened stem, the "eyes" being the much shortened or collapsed lateral branches (APPLEMAN 1). Furthermore, these eyes are located on the tuber in a definitely formed spiral (ARTSCHWAGER 3), comparable to the arrangement on an ordinary leafy twig. In agreement with most stems in young stages, the tubers of most varieties of potato exhibit apical dominance, that is, the ability of the apical bud to prevent the growth of basal buds (APPLEMAN 2). Whatever may be the mechanism by which this dominance of the apical bud is exerted, it is shown in this paper that a treatment with a solution of thiourea prevents the apical bud from completely inhibiting basal buds, and that under certain conditions the direction of dominance may be reversed, so that the apical bud is itself inhibited.

Development of extra buds

From tubers of the Bliss Triumph variety seed pieces were prepared, each weighing about 25 gm. and bearing one eye. They were

[1] Published upon its recent receipt at the expense of the Boyce Thompson Institute for Plant Research.

soaked one hour in a 3 per cent aqueous solution of thiourea, were then rinsed in tap water, planted in soil, and stored in a cool place until sprouting began. The result is shown in fig. 1. It will be noted that out of twenty pieces treated, seventeen or eighteen show the development of more than one sprout per eye, and that in the case of the piece in the upper left hand corner eight sprouts had started.

It is true that not all buds which start will continue to grow, but figs. 2 and 3 show a later development of sprouts from a single eye

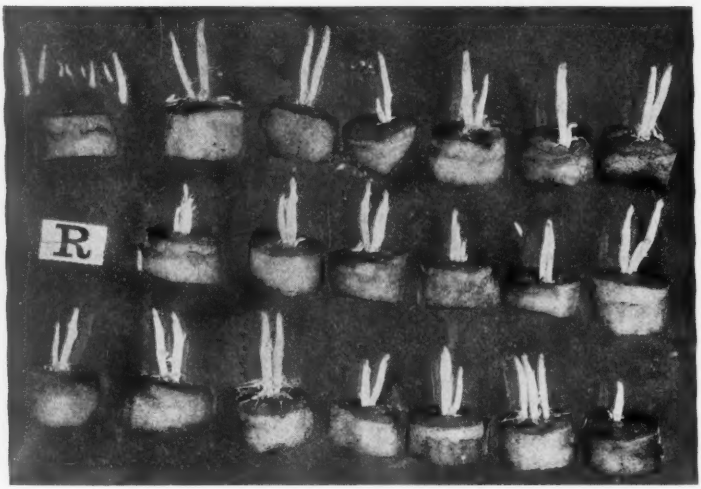


FIG. 1.—Bliss Triumph tubers soaked one hour in 3 per cent solution of thiourea; showing effect of treatment in causing development of more than one sprout per eye.

of another treated tuber. Fig. 3 shows five sprouts that grew from one eye, four of them at approximately the same rate; there is no indication here that one bud is influencing the growth of any other bud, although no doubt the growth of each was being restricted by the lack of sufficient available food in the mother piece. Fig. 3 also shows the development of side branches from a sprout in this early stage of development. With untreated tubers branching does not usually begin until a much later stage of growth. Branching in this case began as soon as the bud emerged from the tuber. It is possible that some of the extra sprouts may be branches from one sprout, but this did not appear to be the case when the cluster of sprouts

was separated. In any event it shows the effect of thiourea upon the course of development of the buds in the eye of the potato.

This multiple sprout effect was obtained with Bliss Triumph, Irish Cobbler, McCormick, and Rural New Yorker varieties. The concentration and time of treatment required to produce it varied with the variety and stage of dormancy, but a suitable range was found to be about 2–4 per cent thiourea, the soaking being continued for one to two hours.

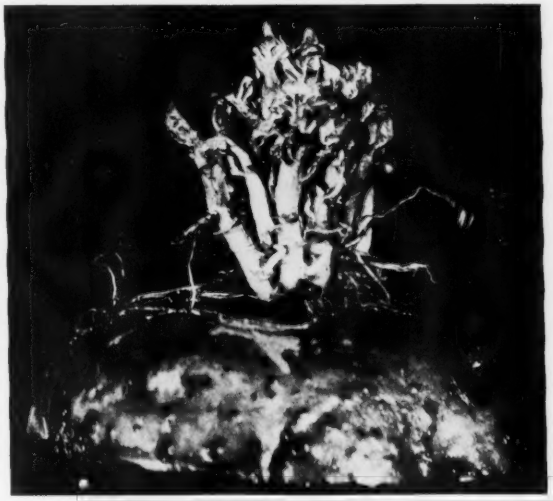


FIG. 2.—Effect of thiourea in inducing formation of several sprouts from one eye of potato (Bliss Triumph); cut tubers soaked one hour in 4 per cent thiourea.

More than 200 chemicals were tried, but thiourea was the only one that consistently forced multiple sprouts without causing rotting of the tubers. As shown in fig. 1, the surfaces of the treated pieces were bright and clear, indicating that no serious injury to the tissue had been caused. In many cases other chemicals caused the development of more than one bud per eye in a small percentage of the pieces treated; but this occurred when the concentration of chemical was high enough to cause considerable injury. In such cases many of the treated pieces rotted either wholly or partly.

The list of chemicals that did not give this multiple sprout response included representatives of various classes of compounds,

both inorganic and organic. Space is not available in this article to publish a complete list of chemicals used, but a few of them may be given, together with a description of the approximate range of concentration and time of treatment. The following were tried by the

FIG. 3.—All sprouts shown grew from one eye of potato (Irish Cobbler); four out of five growing at approximately same rate; inhibition of growth of subsidiary buds by dominant bud prevented; cut tubers soaked one hour in 4 per cent thiourea.

soak method, the time of treatment being one hour:[2] acetamide 0.1–100 gm.; ethyl alcohol 0.3–9 cc. of 95 per cent; boric acid 0.25–10 gm.; bleaching powder (commercial) 2–80 gm.; chloral hydrate 0.25–10 gm.; ethylene glycol 1–25 cc.; ethylene oxide 0.1–10 cc.; furfural 0.5–20 cc.; pyridine 0.01–10 cc.; phenol 0.01–10 gm.; methyl-urea nitrate 1–10 gm.; mercuric chloride 0.1–1 gm.; manganous chloride

[2] The numbers placed after each chemical show the amounts per liter of water.

0.126–12.6 gm.; potassium permanganate 0.0156–15.6 gm.; sodium nitrate 0.85–59.5 gm.; potassium ferrocyanide 0.6–5 gm.; potassium ferricyanide 1–10 gm.; strontium nitrate 3–30 gm.; sodium potassium tartrate 3–30 gm.; ammonium sulphate 33–66 gm.

The following chemicals failed to induce the development of extra buds per eye by the vapor method, using cut tubers:[3] bromoform 0.02 for 3 hours to 0.3 cc. for 24 hours; chlorobenzene 0.06 cc. for 3–24 hours; ether 0.01–2 cc. for 3 hours; ethylene dibromide 0.001 cc. for 3 hours to 0.01 cc. for 24 hours; ethyl nitrite 0.01–0.3 cc. for 3 hours; gasoline 1 cc. for 1–24 hours; methylene chloride 0.5 cc. for 1–24 hours; picoline 0.01–0.3 cc. for 3 hours; acetylene tetrachloride 0.06–0.3 cc. for 16 hours; trimethylene chlorhydrin 0.1–0.5 cc. for 16 hours; toluol 0.06–0.3 cc. for 3 hours.

Injecting the tissue just below the bud with 1 per cent cane sugar and 3 per cent hydrogen peroxide did not force the sprouting of extra buds per eye; soaking in a complete mineral nutrient solution was also without effect.

A special test was made for the purpose of comparing the effect of thiourea and of other "urea" and "thio" compounds. Bliss Triumph dormant tubers from the 1925–26 crop from Bermuda were used. The potatoes were purchased in the Yonkers markets as soon as the Bermuda crop began to arrive. Because of the small size of some of the tubers the cut pieces did not all have only one eye each; some had two eyes. Table I shows the effect of the treatments upon the number of sprouts per seed piece that had appeared above ground after 35 days.

From table I it is seen that only thiourea showed any marked tendency to force the sprouting of more than one bud per seed piece. Urea was ineffective at the concentrations used, although it is possible that stronger percentages for longer periods of treatment would give better results. This should be tested. Ortho-tolyl-thiourea caused prompt sprouting of dormant buds, but double sprouts started from only one seed piece. Di-ortho-tolyl-thiourea was ineffective either in causing early sprouting or in forcing the growth of extra buds.

In these experiments the thiocyanates were next to thiourea in

[3] The numbers following each chemical show the amount of the chemical used per liter of air space in the container in which the treatment was carried out.

the order of effectiveness in respect to multiple sprout formation. In many experiments, not shown in table I, it was noted that double or triple sprouts from single eyes were not uncommon in treated lots with sodium, potassium, or ammonium thiocyanate. The thiocyanates also caused the development of sprouts that were noticeably plumper and fatter than normal sprouts (fig. 4). Occasionally these sprouts would also become twisted or gnarly. These results, although

TABLE I

EFFECT OF THIOUREA AND OF OTHER "THIO" AND "UREA" COMPOUNDS UPON NUMBER OF SPROUTS PRODUCED PER SEED PIECE OF POTATO

CHEMICAL	CONCENTRATION PER CENT	DURATION OF TREATMENT (HRS.)	NUMBER OF SPROUTS PER SEED PIECE	CHEMICAL	CONCENTRATION PER CENT	DURATION OF TREATMENT (HRS.)	NUMBER OF SPROUTS PER SEED PIECE
Thiourea.....	4	1	2.5	Allyl thiourea...	1	1	1.0
Thiourea.....	2	1	1.8	Phenyl thiourea.	*	1	0.5
Thiourea.....	4	1	3.7	Sodium thiocyanate.....	3	1	1.1
Thiourea.....	4	2	4.0				
Thiourea.....	2	1	3.9	Ammonium thiocyanate.....	3	1	1.2
Thiourea.....	2	2	3.9				
Urea.....	10	1	0.6	Sodium thiosulphate.....	8	1	0.1
Urea.....	8	1	0.1				
Urea.....	2	1	0.3	Ammonium thiosulphate.....	8	1	Rotted
Urea nitrate....	3	1	rotted				
Urea nitrate....	1	1	0.2	Potassium cyanide.....	0.1	1	0.5
Urea nitrate....	0.3	1	0.3				
O-tolylthiourea..	*	1	1.1	Dicyandiamide..	3	1	0.0
O-tolylthiourea..	*	2	1.6	Ethylene cyanide	0.2	1	0.3
Di-o-tolyl thiourea.....	*	1	0.2	Check-H_2O.....	.....	1	0.3
				Check-H_2O.....	.....	2	0.9
Di-o-tolyl thiourea.....	*	2	0.9				

* Water-saturated solution.

frequently obtained, could not be produced at will, as was the case with the thiourea treatments, and therefore less emphasis is placed upon them.

Apical dominance

In order to determine the effect upon apical dominance, the tubers were cut lengthwise, into halves or quarters, depending on the size of the tubers. The cut pieces were then soaked in different concentrations of thiourea for different lengths of time, rinsed, and planted in soil and stored in a cool place. After sprouting began they were placed in a greenhouse.

The results of an experiment carried out in this way are shown in fig. 5. The checks in the bottom row show the normal behavior of Bliss Triumph tubers, three out of five pieces only showing one sprout per piece, and this sprout starting from the apical end. In this photograph the pieces are arranged so that the apical end is directed toward the right.

In the two treated lots (lots *G* and *K*, fig. 5), however, it will be noted that the apical end did not dominate the basal end. In most cases sprouts started from two or even more buds on each piece; in some cases buds started nearly as well from the basal end as from the apical. Several examples of multiple sprouts from single eyes are shown in the treated lots.

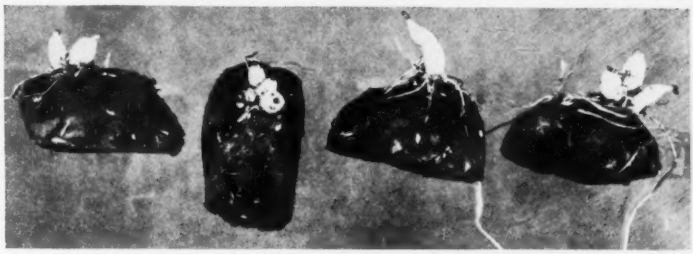


FIG. 4.—Effect of potassium thiocyanate in causing multiple sprouts at eye of potato (Bliss Triumph); note fatness of buds; this result frequently but not always obtained; 3 per cent potassium thiocyanate for one hour.

The behavior of Irish Cobbler and McCormick varieties toward thiourea treatments is shown in fig. 6. The treated lots (lots *M* and *Q*, fig. 6) show complete failure of apical dominance, and also numerous examples of multiple sprouts per eye. At the time the treated tubers were photographed the check tubers had not sprouted (lots *E* and *F*, fig. 6), and the figure therefore shows the condition of the checks six weeks after the picture of the treated lot was taken. The Irish Cobblers show the strong apical dominance of tubers of this variety. This dominance was less marked in the case of the McCormick variety, but the effect of the thiourea on the number of sprouts per eye is clearly shown.

In many cases it was found that the apical buds of tubers treated with thiourea did not grow, but that growth first started from buds

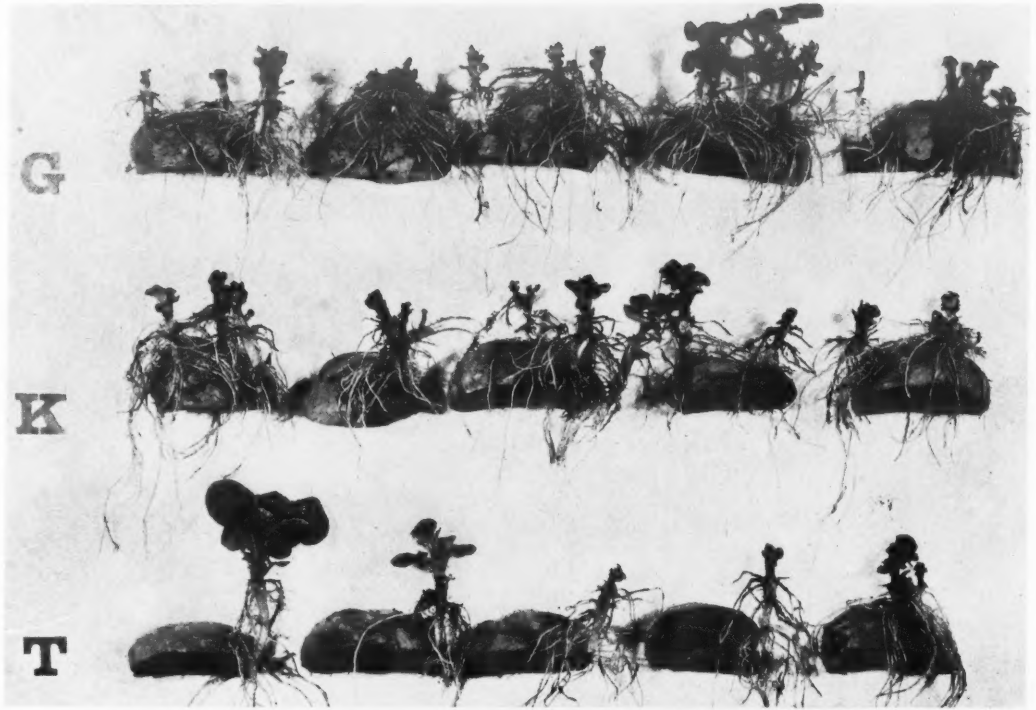


FIG. 5.—Lot *G*, tubers (Bliss Triumph) cut lengthwise into thirds and soaked two hours in 4 per cent thiourea; in each case apical end directed toward right; note absence of apical dominance and formation of multiple sprouts per eye. Lot *K*, same with 2 per cent thiourea for two hours. Lot *T*, check, soaked two hours in water; four out of five seed pieces show apical dominance, and three out of five show only one sprout per seed piece.

toward the basal end. This is shown in fig. 7 (top row, lot *D*), and the two pieces to the right in the second row (lot *H*). The failure

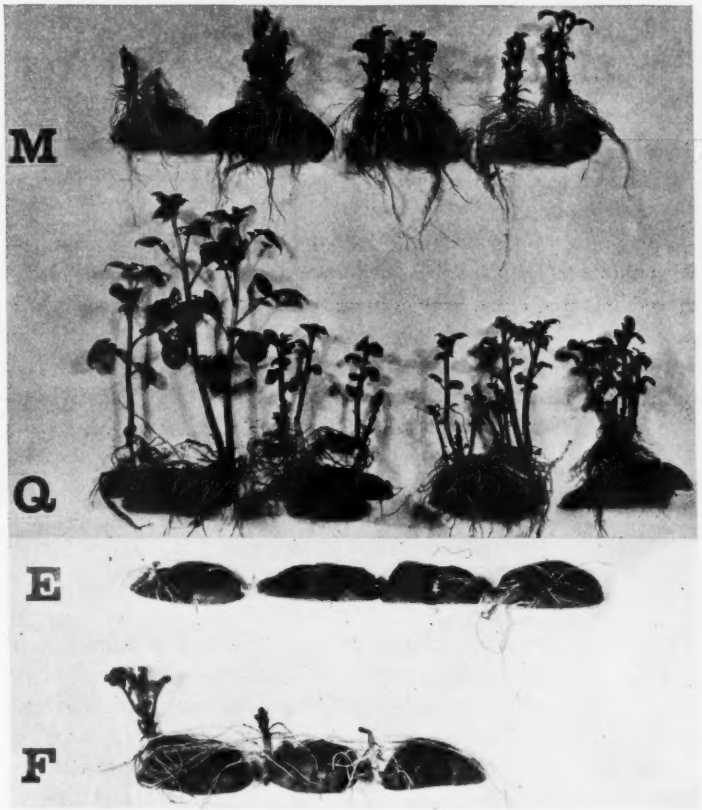


FIG. 6.—Lot *M*, tubers (Irish Cobbler) cut lengthwise into quarters, soaked two hours in 4 per cent thiourea; apical dominance disturbed and multiple sprouts per eye formed. Lot *Q*, same except McCormick variety used. Lot *E*, checks (McCormick) soaked two hours in water. Lot *F*, checks (Irish Cobbler) soaked two hours in water (lots *E* and *F* photographed 44 days after lots *M* and *Q*, but planted at same time).

of the apical buds to grow might have resulted from either of two causes: (1) the apical buds might have been injured or killed and

therefore incapable of growing; (2) the growth of the apical buds might have been inhibited by the growth of the basal buds that had started first.

That the first supposition is incorrect was proved by the fact that such apical buds, when cut off from the tuber and planted separately, sprouted at once. This is shown in fig. 7 (lot *K*, bottom row). The photograph was taken on the ninth day after the apical ends were removed and planted, showing that they had the capacity for prompt growth when removed from the inhibiting influence of the basal buds.

The results shown in fig. 7 indicate that by chemical treatment with thiourea the direction of dominance was reversed, a basally placed bud becoming dominant and inhibiting the growth of the apical bud. A reversal in the direction of dominance was also reported by CHILD and BELLAMY (4) for *Phaseolus*. They found that non-apical buds, forced into growth as a result of physiological isolation from the tip, "in some cases may even inhibit the further growth of the chief tip." The situation here would be analogous if we assume that the thiourea treatment had caused physiological isolation of the apical buds. In what manner this isolation could be brought about by the treatment, however, is not known.

Discussion

The question whether this is a specific response to thiourea cannot be answered by these experiments. It can merely be stated that out of a list of more than 200 chemicals, representing various classes of chemical compounds, thiourea alone showed this effect upon tubers of these varieties. Further experiments would be necessary to determine whether by proper choice of concentration and time of treatment other chemicals could cause similar responses. While it seems unlikely that this is a specific response, it would be desirable to examine the possibility more closely; for if the number of chemicals that can influence bud dominance in this manner can be restricted to one, or only a few, or even to a certain group or type, the study of the cause of dominance will be much simplified.

APPLEMAN (2) expressed the opinion that any treatment that weakened the sprouts destroyed the dominance of the apical bud.

Thus successive removal of sprouts and certain storage conditions resulted in loss of apical dominance. It is true that tubers treated

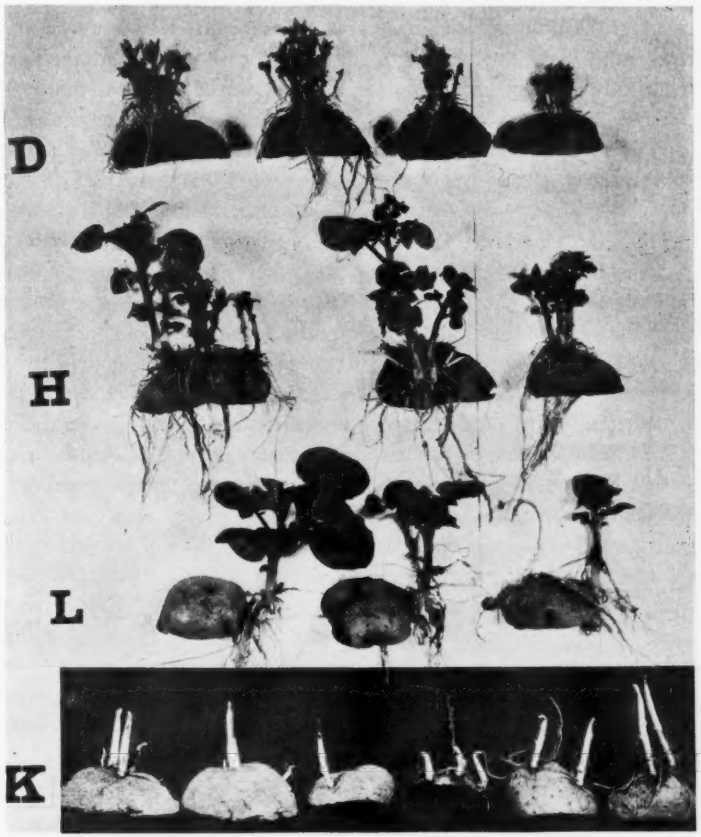


FIG. 7.—Lot *D*, tubers (Bliss Triumph) cut lengthwise into quarters, soaked one hour in 4 per cent thiourea; apical ends directed toward right; note apical buds were not growing, but buds more basally placed started into growth first. Lot *H*, same with 2 per cent thiourea for two hours; apical buds in the two pieces at right not growing. Lot *L*, checks, soaked two hours in water; only apical end growing and only one bud per eye started growth (compare lots *L* and *D*). Lot *K*, apical ends cut off from tubers like those shown in lot *D* and planted separately; inhibited apical buds started growth at once (photograph of lot *K* taken 9 days after planting).

with thiourea under conditions that forced many sprouts per eye did not give strong sprouts. They were thin and spindly. When six or seven were produced from one eye the sprouts were hardly stiff enough to stand upright. This may not be an expression of weakness, however; it may merely mean that there was insufficient food or other growth-promoting substances in the mother tuber to support so many sprouts.

From the standpoint of toxicity it is certain that thiourea is less toxic to potato than many of the substances used. Rotting of the treated pieces was common with most of the chemical treatments, but the thiourea treated lots were notably free from rot. Fig. 1 shows that the surfaces of the treated pieces were clean and free from discoloration or any other evidence of injury. Even when the treatment was severe enough to produce the result shown in fig. 2, the potato tissue was perfectly sound.

It is clear, therefore, that if this disturbance of the normal system of bud correlation in the potato is to be ascribed to a toxic effect, the injury must be caused in some special manner in the case of thiourea. It is believed that factors other than mere injury to the tissue are involved.

At least three theories have been offered to explain why the apical bud is able to prevent the growth of basal buds: (1) that the apical bud produces an inhibiting substance which travels in a basipetal direction and prevents the growth of buds lower down on the stem (ERRERA **6**, REED and HALMA **10**); (2) that there is a higher state of metabolic activity at the tip, and that, as a result of the electric potential set up by this physiological gradient, control is transmitted by way of the protoplasm and in some manner represses the growth of sub-apical buds, but not by the passage of any material substances (CHILD **5**, CHILD and BELLAMY **4**); (3) that not enough nutritive materials are available for all the buds present and that the growing tip causes a flow of materials toward itself and away from the basal buds (GOEBEL **8**, GARDNER **7**). Theories 1 and 3 are also fully discussed by MCCALLUM (**9**).

If inhibition is produced by a substance that is formed by the apical bud, the thiourea effect could be explained on the assumption that the treatment either prevents the apical bud from forming the

substance, or inactivates it in some way after it is formed. The fact that thiourea can nullify this inhibiting effect under certain definite conditions should be of great assistance in experimental work relating to the nature and mode of action of this hypothetical inhibitory chemical.

If the second supposition is correct, then the thiourea may operate, either by preventing the transmission of the stimulus, or by changing the relative metabolic activity of the apical and subapical buds. The results of the experiments, indeed, do show an increase in the rate of growth and therefore of metabolism in the basal buds, but there is little evidence yet to explain how thiourea can produce this change; and there is less to show how thiourea can cause the protoplasm to lose the capacity of transmitting the inhibitive stimulus.

There may be some evidence in favor of the third view, in that thiourea may contribute additional nutrient material by means of which additional buds may develop; but other nutritive substances were not able to induce this response. We would be compelled, therefore, to assume that additional amounts of only certain nutritive substances are necessary to initiate a renewal of growth in the inactive buds. We do not yet know how far the thiourea penetrates into the potato, nor what changes it may itself undergo after entrance into the tissue.

Practical considerations

When the forcing of multiple sprouts by solutions of thiourea was first noted, it was thought that from a practical standpoint this would be an unfavorable response; additional sprouts per seed piece would result in the formation of many small tubers per hill. It was pointed out to the writer by WILLIAM STUART of the United States Department of Agriculture, however, that when potatoes are produced for seed purposes and not for table use, a large proportion of moderately small tubers is desirable, since this size gives greater economy in planting a crop. A chemical treatment that would regulate with security the number of sprouts per hill, therefore, might find application. A further use for such a treatment might be found in the case of varieties that send out too few sprouts per seed piece.

The present high price of thiourea, however, about $15.00 per kilogram, would likely preclude its use in a practical way, and the possibility of using this chemical for such purposes will depend upon finding a cheaper supply of it. It may be that the high price is caused by the methods of purification, and that the impure or unrefined chemical would give equally good results in the potato treatments.

Summary

1. Solutions of thiourea (NH_2CSNH_2) caused the growth of two or more (often four or five) buds from a single eye of the potato.

2. This result was not caused by any other chemical at the concentrations and periods of treatment tried, although more than 200 chemicals representing various classes of chemical compounds were tested.

3. Several "urea" and "thio" compounds were tried, but none showed the consistent results that were obtained with thiourea. Next to thiourea, the most favorable chemicals for this purpose were the thiocyanates.

4. Solutions of thiourea also prevented the apical buds of the tubers from completely inhibiting the growth of basal buds. Certain cases were found in which the direction of dominance was reversed so that the apical buds themselves were inhibited. Such inhibited apical buds when cut off from the tuber and planted separately started into growth at once, after being removed from the influence of previously sprouted basal buds.

5. The relation of these facts to certain theories regarding the cause of the inhibition of basal buds by tip buds is discussed.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH
YONKERS, N.Y.

LITERATURE CITED

1. APPLEMAN, C. O., Physiological basis for the preparation of potatoes for seed. Md. Agric. Exp. Sta. Bull. 212. 1918.
2. ———, Potato sprouts as an index of seed value. Md. Agric. Exp. Sta. Bull. 265. 1924.
3. ARTSCHWAGER, ERNST, Studies on the potato tuber. Jour. Agric. Res. 27:809–836. 1924.
4. CHILD, C. M., and BELLAMY, A. W., Physiological isolation by low temperature in *Bryophyllum* and other plants. Science 50:362–365. 1919.

5. CHILD, C. M., Certain aspects of the problem of physiological correlation. Amer. Jour. Bot. **8**:286–295. 1921.
6. ERRERA, L., Struggle for preeminence and inhibiting stimuli in plants. Brit. Ass. Adv. Sci. Rep. 1904. p. 814.
7. GARDNER, F. E., A study of the conductive tissue of shoots of the Bartlett pear and the relationship of food movement to dominance of the apical buds. Calif. Agric. Exp. Sta. Tech. Paper No. 20. 1925.
8. GOEBEL, K., Regeneration in plants. Bull. Torr. Bot. Club **30**:197–205. 1903.
9. MCCALLUM, W. B., Regeneration in plants. BOT. GAZ. **40**:97–120; 241–263. 1905.
10. REED, H. S., and HALMA, F. F., On the existence of a growth inhibiting substance in the Chinese lemon. Univ. Calif. Pub. Agric. Sci. **4**:99–112. 1919.

CHEMICAL COMPOSITION OF ETIOLATED AND GREEN BERBERIS SPROUTS AND THEIR RESPECTIVE ROOTS[1]

E. R. SCHULZ AND NOEL F. THOMPSON

(WITH TWO FIGURES)

Introduction

The study of the behavior of *Berberis vulgaris* under different conditions was necessary in the investigations of means of eradicating this plant. As a consequence, certain facts were accumulated which bring out some points in regard to the metabolic processes taking place within the barberry. The results herewith reported, on the chemical composition of etiolated and green sprouts and their respective roots, were brought out by an experiment in which the effects of cutting off the tops of the barberry and the subsequent growth of sprouts from the roots were studied in relation to the rate of exhaustion of food material in the roots, and consequently of the death of the plant. In the two kinds of sprouts dealt with, it was noted that such plant constituents as starch, sugars, and ash differed markedly. As such facts dealing with the internal conditions are important to an understanding of the growth activity of plants, the data are presented in this paper.

The formative influence of continuous darkness and of shade on plants has frequently been studied. WEBER (**15**) found in pea seedlings, on the basis of dry weight, more ash in green than in etiolated seedlings grown in a dark cellar. PALLADIN (**5, 6**) reported similar results in regard to ash in wheat and *Vicia Faba* leaves. He also found less protein in the etiolated than in the green leaves. THATCHER'S (**13**) results with potatoes, peas, wheat, oats, and barley showed that shading caused a relative increase in moisture, mineral matter, and nitrogenous matter, and a decrease in carbohydrates. STUTZER and

[1] Cooperative investigation of the Wisconsin State Department of Agriculture, the University of Wisconsin, and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. The writers are indebted to H. B. PARMELE, Agent, Bureau of Plant Industry, for some of the analytical work.

GOY (12) obtained higher ash content in shaded tobacco than in unshaded. SACHS (10) and others have shown that starch may be produced in aerial portions of plants even though these are kept in total darkness. That such darkened portions may store more starch than corresponding portions in the light does not seem to have been reported.

Experiments by SCHLOESING (8) indicated that the relative humidity of the atmosphere may be a very important factor. He grew some tobacco plants under glass jars and others without the jars, and obtained 19 per cent of starch in plants under jars, and 1 per cent of starch in plants grown in the open. He explained the high percentage of starch in the plants under the glass jars as being due to reduced transpiration rather than to reduced light. The bearing of these results upon the experiments reported in this paper will be discussed later.

Experimentation

MATERIAL

The analytical studies here recorded were conducted on four lots of *Berberis* tissues, namely, green sprouts and their roots, and etiolated sprouts and their roots. The plants used for the experiments (fig. 1) were growing in two localities with slightly different soil conditions, but approximately the same climatic conditions. The first locality was near Marshall, Dane County, Wisconsin. The soil was clay loam rich in humus, and the plants were large escaped bushes growing on the edge of a woodlot where birds and cattle evidently had dropped the seeds. Eight plants were selected at this place, four on May 19 and four on May 26, 1923. The tops were cut off close to the ground, and the stumps and roots of half of the number were covered with wooden boxes, lined on the inside with roofing paper to insure complete darkness; the other four stumps were left uncovered. Composite collections of roots from plants growing in the vicinity of the selected plants were taken at the beginning of the experiments. These samples were analyzed and used for subsequent comparison with identical storage products in the roots of the decapitated plants.

The second locality was near Gurnee, Lake County, Illinois. The soil here was a brown silt loam, very rich in humus. The bushes were

escaped from cultivation and were growing in a woodlot. The tops of 34 plants were cut off close to the ground on April 14, 1924, and over the stumps and roots of half of the number were constructed light-proof housings, 3 ft. square, made of stakes and roofing paper.

FIG. 1.—Type of *Berberis* plant used in these experiments.

The remaining 17 were left uncovered. A composite collection of roots from adjacent plants was collected and analyzed as was done at Marshall, Wisconsin.

The coverings at both Marshall and Gurnee proved capable of excluding light, as the developing sprouts showed no green; they

were always bright yellow, which is typical of *Berberis* and due to an alkaloid present in the tissues. Ventilation under the coverings was rather poor, but the growth of the sprouts under such cover was not less vigorous than in the open.

METHODS

COLLECTION AND PREPARATION OF SAMPLES FOR ANALYSIS.—Several collections of sprouts and roots were made, as indicated in tables I–III. That portion of the samples intended for analysis in the dry state was always cut into small pieces, and within one hour of the time of collection it was given a preliminary drying for one-half hour at 98° C. to stop metabolic processes. The samples collected at Marshall were transported to Madison, where the laboratory was located, before giving them this preliminary drying. For the samples collected at Gurnee, an oven was provided near by where this drying was performed. Later, all the samples were dried at 65° C. for 10 hours, and then ground to a powder that passed through a 100-mesh sieve. From this ground material the carbohydrates were determined, since LINK (3) found that they were altered in tissues dried at higher temperatures. For the moisture determination, a 2 gm. sample of this ground material was dried to constant weight at 102° C. The portion of the samples intended for analysis in the fresh state was carefully wrapped in oiled paper and transported to Madison as rapidly as possible. The material was then immediately chopped into small pieces and ground in a mortar with quartz sand and extracted with water, as described by TOTTINGHAM, SCHULZ, and LEPKOVSKY (14).

ANALYSIS.—The Kjeldahl-Gunning method was used for nitrogen determination. Sugars and sugar equivalents of starch and hemicellulose were determined by the SHAFFER-HARTMANN (9) method. The sugars from the dried tissues were extracted with 90 per cent alcohol; the alcohol was then evaporated at 50°–55° C. under reduced pressure. From one fraction of this extract reducing sugars were determined, and the other fraction was hydrolyzed with 2.5 per cent HCl for 45 minutes previous to total sugar determination. Neutral lead acetate was used for clarification of the sugar-containing solutions. Saliva was used for removing starch from the sugar-free tis-

sue, preceding hydrolysis, and the starch was hydrolyzed by the action of 2.5 per cent HCl for 2.5 hours. The tissue from which the starch had been removed was hydrolyzed also with 2.5 per cent HCl for 2.5 hours, and the hydrolyzable fraction was designated as hemicellulose. Pentosans were determined from this hydrolyzable fraction, as well as from the residue left after the hydrolysis. The method of the Official Agricultural Chemists was used for pentosan determination.

The results of analysis, as shown in table II, indicate that the concentration of acid, and the duration of hydrolysis preceding total sugar determination were too great. The low total sugar content in the sprouts, which in 3 cases out of 12 is actually lower than the reducing sugars, must be due to the destruction of simple sugars such as levulose, which, according to BROWN and MORRIS (2), PARKIN (7), SPOEHR and MCGEE (11), and others, are found in abundance in the succulent parts of plants. The comparative ease with which the ketose sugars are affected by mineral acids also supports this assumption.

RESULTS

SAMPLES FROM MARSHALL, WISCONSIN.—Samples were collected on five different dates, as shown in table I. Each collection consisted of green and etiolated sprouts and the roots from which they grew. The percentages of moisture, ether extract, total sugar, starch, hemicellulose, and total nitrogen of each sample were determined. There were noticeable differences in the quantity of these constituents between the green and etiolated sprouts. Moisture and starch were lower in every case in the plants grown in the light; also nitrogen and sugar were lower in four cases out of the five. The ether extract and hemicellulose were the only substances determined that were approximately the same in the two sets of samples. With the roots, on the other hand, no definite differences could be determined, nor did the later collections show any appreciable decrease over the earlier ones.

SAMPLES FROM GURNEE, ILLINOIS.—The samples collected were similar to those from Marshall, Wisconsin, except that considerably greater numbers of plants were involved, and therefore they were more nearly representative. Additional substances also were deter-

mined in the analysis (tables II, III). The results agreed very closely with those of the former analyses. Only in the cases of ether extract and total nitrogen were there differences. In every case the former was higher in the green sprouts than in the etiolated ones, while the

TABLE I

ANALYSIS OF BERBERIS PLANTS GROWN IN LIGHT AND IN DARKNESS AT MARSHALL, WISCONSIN

PORTION OF PLANT	PLANTS GROWN IN	DATE OF COLLECTION	PERCENTAGE MOISTURE IN FRESH TISSUE	PERCENTAGE DRIED TISSUE				
				Ether extract	Total sugar	Starch	Hemicellulose	Total nitrogen
	Four plants cut down and half of the stumps covered May 19, 1923							
Sprouts	Light	June 18	81.00	6.66	3.49	3.81	13.65	2.58
	Dark	June 18	95.76	7.95	3.85	5.86	11.23	3.53
	Light	July 9	88.58	7.55	3.44	3.50	13.18	1.12
	Dark	July 9	96.20	4.60	8.31	6.40	10.78	2.45
Roots	Light	May 19	48.13	0.70	5.74	13.81	16.34	0.78
	Light	July 9	52.34	0.90	4.03	10.64	18.87	0.64
	Dark	July 9	54.70	1.06	3.63	16.28	15.88	0.73
	Four plants cut down and half of the stumps covered May 26, 1923							
Sprouts	Light	June 26	90.96	6.95	3.64	3.53	10.10	3.01
	Dark	June 26	94.91	7.43	4.70	4.37	11.48	2.77
	Light	July 19	87.09	6.03	1.76	2.60	13.75	2.28
	Dark	July 19	89.22	4.56	5.35	4.75	13.09	2.48
	Light	August 22	79.15	6.38	2.68	2.55	14.25	1.43*
	Dark	August 22	85.30	2.79	2.05	4.27	14.65	1.63*
Roots	Light	May 26	50.70	0.70	3.17	12.51	17.51	0.68
	Light	June 26	58.27	0.84	3.81	4.21	17.72	0.66
	Dark	June 26	56.65	0.79	3.99	4.35	17.35	0.65
	Light	July 19	56.49	1.52	3.23	6.00	19.36	0.72
	Dark	July 19	49.93	1.52	2.44	11.28	17.73	0.63

* Water-soluble nitrogen in the fresh sprouts was determined for the collection of August 22. The sprouts in the light had 0.47 per cent and in the dark 1.09 per cent, based on the dry weight.

nitrogen was about equally divided. Of the additional substances determined, reducing sugar (both from dried and fresh tissue), sucrose, water-soluble nitrogen, and ash were consistently higher in the sprouts grown in the dark than in those grown in the light. Pentosans were slightly higher in the green sprouts than in the etiolated ones.

At Gurnee, as at Marshall, there were no consistent differences in the substances found in the roots, nor was there an appreciable

TABLE II

ANALYSIS OF BERBERIS PLANTS GROWN IN LIGHT AND IN DARKNESS AT GURNEE, ILLINOIS

PLANTS GROWN IN	DATE OF COLLECTION	PERCENTAGE MOISTURE IN FRESH TISSUE	PERCENTAGE DRIED TISSUE							
			Ether extract	Sugar		Starch	Hemicellulose	Pentosans	Total nitrogen	Ash
				Reducing	Total					
			Sprouts							
Light.....	June 18	85.00	.....	.....	.....	.....	.....	.....	3.32	.....
Dark.....	June 18	87.00	.....	.....	.....	.....	.....	.....	2.90	.....
Light.....	July 2	84.36	2.83	2.16	3.02	4.07	11.30	5.20	3.12	5.65
Dark.....	July 2	87.47	1.25	4.52	5.72	4.62	8.95	2.58	3.32	9.90
Light.....	July 25	84.17	3.65	2.90	1.54	2.95	9.55	6.98	3.34	5.90
Dark.....	July 25	83.97	1.20	2.46	2.78	3.32	9.85	7.83	3.70	7.60
Light.....	August 16	80.57	2.85	2.46	2.58	2.15	10.85	7.43	2.67	5.40
Dark.....	August 16	84.65	1.05	3.20	3.90	2.95	9.55	7.38	3.03	10.20
Light.....	September 16	77.31	1.92	2.10	1.74	3.17	10.85	7.45	2.28	5.85
Dark.....	September 16	83.51	1.00	5.84	5.64	4.00	10.85	6.18	2.21	13.55
			Roots							
Light.....	April 14	55.00	0.38	1.30	3.70	9.95	11.75	8.97	0.82	9.50
Light.....	July 2	54.96	0.50	1.02	3.38	9.55	14.00	8.03	1.05	13.55
Dark.....	July 2	58.46	0.60	0.71	3.44	8.42	14.75	6.66	0.96	10.95
Light.....	July 25	56.51	0.27	1.54	2.72	10.05	13.85	8.42	0.94	7.05
Dark.....	July 25	55.83	0.07	1.24	2.28	6.70	15.20	9.28	0.86	5.45
Light.....	August 16	58.29	0.10	1.42	3.90	3.77	15.65	8.93	0.73	10.35
Dark.....	August 16	44.77	0.10	1.54	4.44	6.62	14.60	8.01	0.88	10.75
Light.....	September 16	53.27	0.00	1.54	3.15	7.07	16.55	8.87	0.75	4.25
Dark.....	September 16	52.97	0.03	1.54	3.60	8.12	15.80	8.20	0.80	5.70

lessening of the stored materials in the later collections over the earlier ones, even though a considerable quantity of sprouts had developed from them in the interval.

Discussion

A survey of the data presented indicates that sprouts from the roots of *Berberis vulgaris* differ markedly in chemical composition when grown in the light and under conditions which excluded light. In the latter case photosynthesis is absent, and growth and respiration of the sprouts are supported entirely by the translocation of

TABLE III

ANALYSIS OF WATER-SOLUBLE CONSTITUENTS OF FRESH TISSUE OF BERBERIS SPROUTS GROWN IN LIGHT AND IN DARKNESS AT GURNEE, ILLINOIS .

SPROUTS GROWN IN	DATE OF COLLECTION	PERCENTAGE DRIED TISSUE			
		Reducing sugar	Sucrose	Total sugar	Nitrogen
Light	June 18	3.08	0.45	.....	1.19
Dark	June 18	7.94	1.47	.....	1.56
Light	July 2	3.30	0.41	.....	1.26
Dark	July 2	6.70	1.90	.....	1.67
Light	July 25	3.77	0.39	.....	1.14
Dark	July 25	5.31	0.56	.....	1.25
Light	August 16	1.51	0.40	2.42	0.70
Dark	August 16	5.23	0.78	6.24	1.33
Light	September 16	1.16	0.74	3.20	0.83
Dark	September 16	6.15	2.33	8.07	0.89

products from the roots, which are rich in carbohydrates and rather rich in nitrogenous materials. Sprouts growing in the light, on the other hand, may draw upon the reserve materials stored in the roots as well as synthesize new materials. From this it would appear that the etiolated sprouts should be less abundantly supplied with carbohydrates than the green sprouts.

The results show, however, that carbohydrates, such as reducing sugars, total sugars, and starch were higher in etiolated than in green sprouts. Hemicellulose and pentosans were lower, as a rule, in the etiolated sprouts (fig. 2). These results, in this case from sprouts of woody plants, differ in some respects from results reported in the literature and obtained with plants grown from seeds. Various workers have reported the starch, or carbohydrate content

in general, as being lower in etiolated than in green plants. An exception is the work with tobacco reported by SCHLOESING and previously mentioned. High starch content was obtained in this case in plants with reduced transpiration caused by belljars. The tight housings made of roofing paper, under which the etiolated sprouts were grown, might have caused reduced transpiration, and thus resemble the belljars in the experiments of SCHLOESING. The question, however, of how reduced transpiration causes the accumulation of starch, if at all, is not clear and calls for further experimentation.

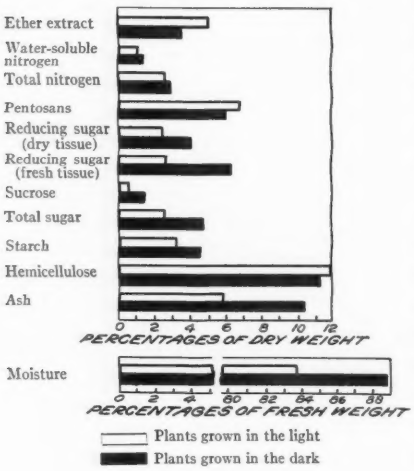


FIG. 2

FIG. 2.—Average percentages of all determinations of the various constituents of *Berberis* sprouts growing in light and in darkness.

More obvious, although not more definite, explanation in regard to high carbohydrate content in the etiolated sprouts is suggested by the growth habit of the plant under natural conditions. It is well known that it forms stolons which may serve as a storage place for excess nutritive materials. The condition of darkness might have changed the sprouts to stolons so far as composition is concerned. Experimental physiology supports this assumption in regard to the change of form, although no chemical composition of such forms is reported. PALLADIN (4) cites experiments in which above-ground tubers were developed on potato vines, and aërial rhizomes on stems of *Stachys palustris* and other plants by means of darkening a section of these plant parts.

The high content of soluble nitrogen found in etiolated sprouts (table III, fig. 2) is in agreement with the results of other investigations, such as those of SPOEHR and MCGEE. This might be due to amino acid accumulation when the plants were grown in darkness,

the amino acids acting as catalysts in increasing carbohydrate catabolism. Even if this were true, however, the carbohydrate translocation and storage were greater than the consumption.

The earlier workers found etiolated plants low in ash content. More recent investigations have shown etiolated or partly shaded plants higher in ash than plants grown in full light. The results with *Berberis* show the etiolated sprouts consistently higher in ash than the green, being as much as two and three times as high (table II, fig. 2). No satisfactory explanation of such behavior suggests itself. It may, however, be due to the etiolated plant having less stable compounds than the green, and these being capable of uniting with more inorganic constituents. The inorganic salts may also be a factor in aiding the metabolic processes in the absence of light, as BAUDISCH (1) has shown. He demonstrated that some salts, for example $Fe(OH)_2$, may cause oxidation, reduction, and synthesis of organic substances, thus in some respects taking the place of light in plant metabolism.

The roots of the etiolated and green sprouts, respectively, did not show marked differences or even appreciable decrease in nutritive storage materials due to translocation and utilization of such materials by the developing sprouts. The duration of the experiment was not sufficient markedly to affect the rich storage supply of the root.

STATE CAPITOL ANNEX
MADISON, WIS.

LITERATURE CITED

1. BAUDISCH, O., The influence of light on inorganic matter and life processes. Jour. Ind. and Eng. Chem. **15**:451–454. 1923.
2. BROWN, H. T., and MORRIS, G. H., A contribution to the chemistry and physiology of foliage leaves. Jour. Chem. Soc. London **63**:604–677. 1893.
3. LINK, K. P., Effects of the method of desiccation on the carbohydrates of plant tissue. Jour. Amer. Chem. Soc. **47**:470–476. 1925.
4. PALLADIN, V. I., Plant physiology. Eng. transl. ed. by B. E. LIVINGSTON. pp. 320. Philadelphia. 1918.
5. PALLADIN, W., Aschengehalt der Etiolirten Blätter. Ber. Deutsch. Bot. Gesells. **10**:179–183. 1892.
6. ———, Respiration des Feuilles Vertes and des Feuilles Étiolées. Rev. Gen. Bot. **5**:449–473. 1893.

7. PARKIN, J., The carbohydrates of the foliage leaf of the snowdrop (*Galanthus nivalis* L.) and their bearing on the first sugar of photosynthesis. Bioch. Jour. **6**:1–47. 1912.
8. SCHLOESING, TH., Vegetation comparée du tabac sous cloche et à l'air libre. Compt. Rend. **69**:353–356. 1869.
9. SHAFFER, P. A., and HARTMANN, A. F., The iodometric determination of copper and its use in sugar analysis. Jour. Biol. Chem. **45**:349–390 (parts I and II). 1921.
10. SACHS, J. VON, Lectures on the physiology of plants. Eng. transl. by H. M. WARD. pp. 14–836. Oxford. 1887.
11. SPOEHR, H. A., and MCGEE, J. M., Studies in plant respiration and photosynthesis. Carnegie Inst. Wash. Bull. 325. pp. 98. 1923.
12. STUTZER, A., and GOY, S., Der Einfluss der Beschattung des Tabaks auf verschiedene Bestandteile der Blätter. Biochem. Zeits. **56**:220–229. 1913.
13. THATCHER, R. W., The influence of shade upon the composition of plants. Jour. Ind. and Eng. Chem. **1**:801–802. 1909.
14. TOTTINGHAM, W. E., SCHULZ, E. R., and LEPKOVSKY, S., The extraction of nitrogenous constituents from plant cells. Jour. Amer. Chem. Soc. **46**:203–208. 1924.
15. WEBER, R., Über den Einfluss farbigen Lichtes auf die Assimilation und die damit zusammenhängende Vermehrung der Aschenbestandtheile in Erbsen-Keimlingen. Landw. Versuchs-Stat. **18**:18–48. 1875.

PHYSIOLOGICAL INVESTIGATION OF BLACK HEART OF POTATO TUBER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 347

WARD B. DAVIS

(WITH SEVEN FIGURES)

Sometime prior to 1913, a blackening of the inner tissues of the potato during shipment was reported to experiment stations in northern United States. This condition, which was later found to be produced also in storage, is now known as black heart. Black heart is an abnormal discoloration of the central regions of the potato, in which there is a series of successive color changes from normal to pink, brown, and black on exposure to the air.

BARTHOLOMEW (**6**) made a preliminary report on black heart in 1913, and in 1914 (**7**) worked out the cause of the color change. No pathological organism was found. He believed that high temperatures maintained to prevent freezing during shipment caused increased respiration of the potato, and assumed that the increased respiration would naturally lead to an accumulation of carbon dioxide and a lack of oxygen in the central tissues. He thought that this accumulation of the products of respiration and lack of oxygen brought about the death of the cells, by which a greater amount of tyrosine was made available to be acted upon by the enzyme tyrosinase, to produce black melanic substances. Under laboratory conditions he produced the disease at 38°–44° C. in 15–24 hours. The optimum temperature for its development was 42°–44°.

Somewhat more recently, STEWART and MIX (**24**) have investigated the production of black heart, with the object of devising practical methods of preventing spoilage in shipping and storage. They showed that high temperature was not a necessary condition for producing the abnormality, since they produced it by confining the tubers in sealed jars for about 20 days at 12°–15° C. The conditions to which these tubers were subjected were similar to those resulting from deep piling and lack of ventilation. For this reason it

seemed probable that there were two important contributing factors in producing black heart in shipping and storage, high temperatures and lack of ventilation.

The present study was undertaken to determine some of the physiological changes which precede or accompany such profound changes in the cells of the interior. This was accomplished by producing the disease in the laboratory, and during its progress recording the following changes in (1) ratio of CO_2 to O_2 in intercellular spaces; (2) electrical conductivity of tissues; (3) catalase activity; (4) H-ion concentration.

Methods

In preliminary studies the methods later to be described were worked out separately. The individual variation of the potatoes in their susceptibility was so great that it soon became apparent that determinations made on the same tissue would more accurately indicate the changes involved. For this purpose, disease-free potatoes of the Rural New Yorker variety grown on peat soil were secured from a commercial firm in Minnesota. These potatoes were planted May 20, harvested September 20, pitted with dirt and straw until October 27, and then removed to a root cellar until the date of shipping, November 20, 1923. On their receipt at the laboratory they were stored at 17°–18° C., and the determinations were made in the ensuing three weeks.

Before these determinations were carried out, respiration of the normal tubers was determined. From 16 to 18 clean, well shaped potatoes were numbered, weighed, divided into two lots of approximately equal weight, and the respirations determined in the type of apparatus used by JOHNSTONE (**12**). The water bath used was electrically heated and regulated at 25° C. All results were reduced to standard conditions of 760 mm. pressure and 0° C. The respiration for each of the three series of tubers run was determined in the same way.

After the respiration had been determined, each lot of 8 or 9 tubers was placed in a 5 l. Pyrex desiccator provided with KOH, a thermometer, and two connections,—one to a graduated cylinder of pure oxygen, and the other to a manometer for keeping the pressure constant. The negative pressure developing in the oxygen cyl-

inder as the gas was withdrawn was constantly relieved by water from a water reserve to which the cylinder was attached. The type of water bath employed by MAGNESS and DIEHL (**16**) in apparatus for the determination of respiration of apples was used. The desiccator, oxygen cylinder, and water reserve were kept in an electrically heated and regulated oven at 45° C. Three series of two lots each, consisting of 16–18 potatoes to the series, were run. To avoid unusually large containers and to keep the tubers under as nearly the same conditions as possible, it was necessary to use the two lots to each series. Removal from the first lot began at the end of the first hour, while removal from the second lot was begun at the end of the eighth or ninth hour. Determinations were started immediately after removal.

GAS ANALYSIS.—By means of a cork borer, a sample 22 mm. in diameter was cut at right angles to the long axis of the potato, at about an equal distance from each end. Buds were avoided. From this sample the intercellular gases were extracted by means of an apparatus which MAGNESS (**15**) has described. The gases were collected over mercury, and analyzed for CO_2 and O_2 in the Bonnier-Mangin apparatus.

CONDUCTIVITY.—From the cylinder of tissue used for gas extraction, a second cylinder of smaller diameter was cut with a cork borer. About equal lengths were cut from the ends, so that the cylinder of interior tissue remaining was 13 mm. long and had a volume of approximately 1 cc. This cylinder was immediately placed between platinized platinum electrodes of a conductivity cell specially devised for this purpose (fig. 1). That the movable mercury electrode (*a*), which weighed 63.73gm., always made good contact with the tissue was shown by the fact that the cylinder of tissue was often found adhering to it when it was lifted. The measurement of resistance was made in the usual manner, by making the conductivity cell one branch of a Wheatstone bridge. A Leeds and Northrup Kohl-

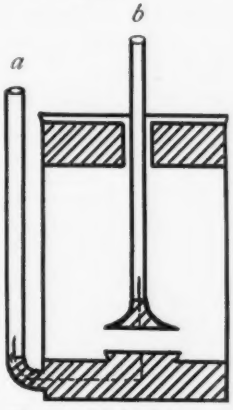


FIG. 1.—Conductivity cell

rausch circular slide wire bridge and a four dial resistance box were used. A microphone hummer, giving a thousand cycle frequency, was the source of the alternating current. Readings were taken at the middle of the bridge by balancing the known resistance against the unknown. Baldwin head phones were used for detecting the point of minimum sound. While the apparatus for determining resistance was quite accurate, the great differences in resistance between normal and abnormal tissue made unnecessary any attempt to secure the accuracy that can be obtained for solutions of electrolytes.

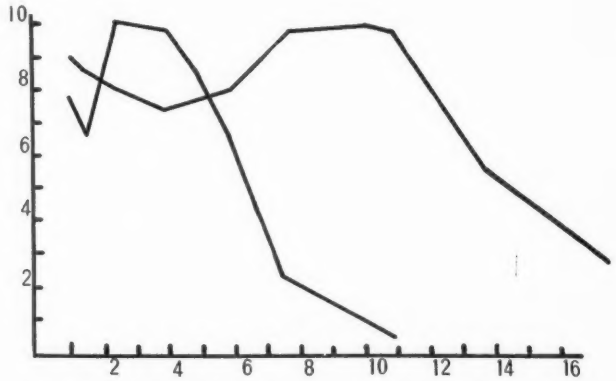


FIG. 2.—Conductivity of two potatoes (*A* and *B*) determined by inserting electrodes in each tuber separately; axes as in fig. 4.

Another method for getting a direct measurement of conductivity of the tissue was also used. While this method could not be used in the series just mentioned, it gave much more accurate indications of the course of the breakdown in a single tuber.

Two parallel pieces of steel 13 mm. apart and rigidly fastened together were thrust to the center of the potato from the stem end. Into the two slits thus formed two platinum foil electrodes with mercury connections were thrust, and sealed in with melted paraffin. The tuber was supported in a desiccator provided with a thermometer and a small opening to allow for the expansion of gases. The desiccator was then placed in an electrically heated and regu-

lated oven at 45° C. The electrodes were put in the circuit of the Wheatstone bridge in place of the conductivity cell, and readings made at will. Results obtained by this method are given in fig. 2.

CATALASE ACTIVITY.—As soon as the cylinder of tissue had been used for conductivity determinations, it was placed in a weighing bottle, weighed, and used for the determination of catalase activity. Essentially the same method as that which APPLEMAN (1) has described was followed. Calcium carbonate was used for neutralizing the acidity of the ground tissue and also of the "dioxogen." The amount of O_2 released from 10 cc. of standardized dioxogen in 10 minutes by 1 gm. of tissue was reduced to 0° C. and 760 mm. pressure.

HYDROGEN-ION CONCENTRATION CHANGES.—The portion of the tuber left after samples for gas analysis and conductivity were obtained was used for the determination of H-ion. CLARK and LUB indicators were applied directly to the tissues by a method similar to that described by ATKINS (4), PRIESTLY (18), and others. Since the indicators were applied before coloration of the tissues began there is no interfering color, and except for the vascular rings and buds which seem to be acid, the tissues are very uniform. Comparison of the injured with the uninjured areas was easily made in this way. Brom cresol purple and methyl red were used in each of the series of determinations. The former proved less satisfactory than the latter, especially at a P_H around 5.6–5.7.

TEMPERATURE.—The possibility that temperature effects, aside from causing increased respiratory activity, might be a factor, led to the use of the change in weight method which STILES and JØRGENSEN (25) have used to test the water-holding power of potato disks in relation to temperature. They found a critical point for water retentivity to lie somewhere between 30–40° C. Their methods were closely followed in determining this point a little more accurately for the potatoes used.

Data

PRELIMINARY STUDY.—An illustration of some of the results of preliminary conductivity studies is given in table I. This lot of potatoes, unconfined in jars, was placed in the oven at 45° C. The results show the variation in susceptibility due to size and other fac-

tors. Marked variation in resistance of the interior samples during the early stages of black heart is due to the impossibility of always including in the samples the affected tissues. In spite of this, even in preliminary studies where the conditions were not carefully controlled, the difference in conductivity between the interior and exterior tissues is quite marked.

TABLE I

CONDUCTIVITY OF INTERIOR AND EXTERIOR TISSUES OF POTATOES DURING PROGRESS OF BLACK HEART INJURY

No.	Weight in gm.	Loss in weight	Hours in oven	Ohms resistance		Condition of sample
				Exterior	Interior	
4...	110.00	.....	0	6000	6000	Not colored
8...	105.60	.....	3	5000	5000	Not colored
1...	132.10	0.43	6	6000	1800	Slightly colored
11...	142.65	0.47	10	7000	960	Slightly colored
			10	5700	1100	Slightly colored
2...	121.40	0.39	10	5300	450	Quite colored
			10	5000	1800	Slightly colored
			Average 10	5750	1078	
9...	182.93	0.68	13	6000	280	All colored
			13	5160	400	All colored
6...	140.37	0.59	13	5400	540	All colored
			13	4400	510	All colored
			Average 13	5240	433	
3...	167.20	0.800	16	3540	240	All colored
			16	5300	1160	Slightly colored
5...	142.18	1.780	16	4200	330	All colored
			16	6500	290	All colored
			Average 16	4885	505	
10...	171.85	1.03	19	5530	270	All colored
			19	4000	360	All colored
7...	132.75	0.95	19	5800	300	All colored
			19	4100	405	All colored
			Average 19	4882	334	

RESPIRATION.—The average results for the respiration of two of the three series run are given in table II. The determinations for the third series are not included, because of difficulties with the mechanism for temperature control of the water bath. Other tubers re-

served from the same lot did not sprout in storage at 17°–18° C. until two months later. They were partially after-ripened and heavily suberized at the time of the determinations.

TABLE II

RESPIRATION

	MG. CO_2 PER KG. PER HOUR	MG. O_2 PER KG. PER HOUR	CO_2/O_2
Average of four lots.....	12.1217	9.9630	0.618

GAS ANALYSIS.—The ratios of CO_2 to O_2 in the intercellular spaces of the potato vary with the conditions of storage, such as temperature and depth of piling, and with the age of the tubers. The

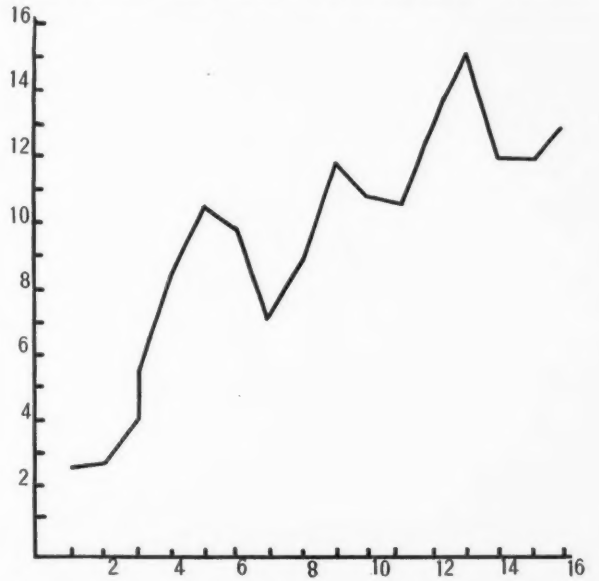


FIG. 3.—Ratio of CO_2/O_2: vertical axes represent ratio; horizontal axes represent time in hours.

amount of O_2 in the untreated tubers used in these experiments was 10–11 per cent, while that of the CO_2 was 5–6 per cent, making a ratio of much less than one. As shown in fig. 3, during the first hour

in the oven at 45° C. this ratio had increased to over two, in spite of the abundant O_2 in the atmosphere of the desiccator. Since the tubers removed from storage at 17°–18° C. cooled the interior of the desiccator somewhat, the temperature of the air surrounding the tubers was around 35°–36° at the beginning, and did not reach 45° until 5 or 6 hours later. At the end of 16 hours the percentage of CO_2 had increased to about 50, and the oxygen had correspondingly decreased to below 4 per cent. Since the samples from which the gases were extracted included a portion of the exterior as well as the interior tissues, the percentage of CO_2 in the interior must have reached far above 50.

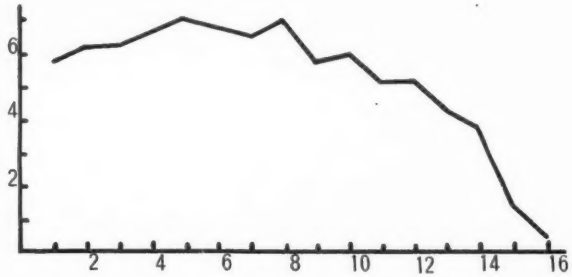


FIG. 4.—Average conductivity of three series: vertical axes represent resistance in thousands of ohms; horizontal axes represent time in hours.

CONDUCTIVITY.—OSTERHOUT'S (17) work on conductivity suggested the use of an accurate expression of the rate and degree of breakdown in the tuber during the progress of the disease. In spite of the variations already mentioned, the results of both the methods compare favorably in showing the course of the breakdown. The early rise of resistance which figs. 2 and 4 show took place at a period when the temperature of the potato must have been increasing, and when one would naturally expect the resistance to be falling. If tubers were cut open at the point where there is an unmistakable sharp fall in resistance and exposed to the air, the discolorations would appear in the central tissues. This fall no doubt is due to the increased permeability following the death of the cells in the region affected.

CATALASE ACTIVITY.—Before the problem was undertaken, the work of APPLEMAN (**2**, **3**) had suggested a correlation between catalase and respiration. The ease with which catalase is determined makes this suggestion an important one. If catalase were correlated with respiration, the curve for catalase activity as given in fig. 5 should rise, at least at the beginning, but such is not the case. The curve for the decrease of O_2 in the intercellular spaces will more nearly parallel the curve for catalase activity.

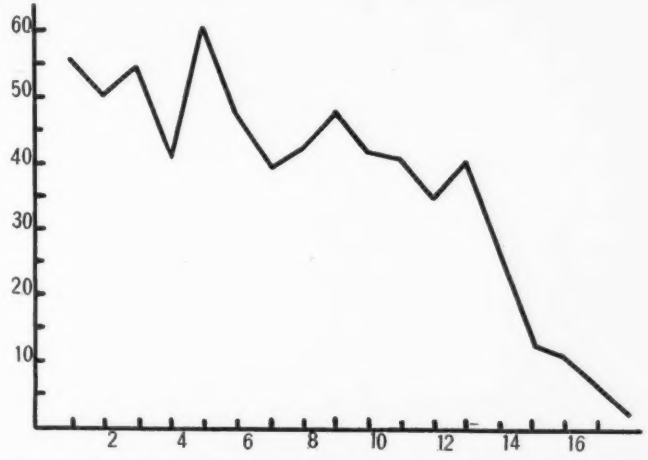


FIG. 5.—Catalase activity: vertical axes represent cc. of oxygen; horizontal axes represent time in hours.

HYDROGEN-ION CONCENTRATION CHANGES.—Although the H-ion concentration change revealed by the indicator method is small, there is an unmistakable increase, as indicated by fig. 6. Working under the best condition for detecting local changes, none were found. Within the limits of the method little importance can be attributed to the influence of the H-ion in causing localization of the affected tissues.

TEMPERATURE.—While it is by no means proved that heat is a factor in the production of black heart at high temperature, the abrupt change in the water-holding power of potato disks at about 38° C. (fig. 7) is quite suggestive. A slight loss of water with in-

creased permeability might be sufficient to hinder gaseous exchange by clogging the very small intercellular spaces. Very extensive clogging would hinder the extraction of gases, however, and this does

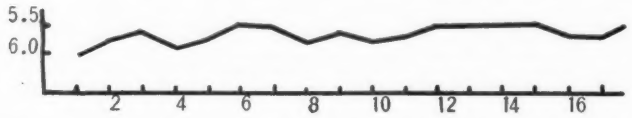


FIG. 6.—Hydrogen-ion concentration: vertical axes represent P_H value; horizontal axes represent time in hours.

not occur, except when tubers are left for much longer periods than those used in these experiments.

The pioneer work of DEVAUX (11) has left no doubt regarding the existence of intercellular spaces in many fleshy storage tissues, including the potato.

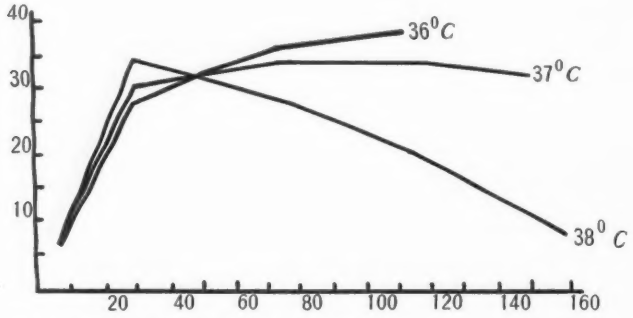


FIG. 7.—Water-holding power of potato disks: vertical axes represent percentage of water intake based on original weight of disks; horizontal axes represent time in minutes.

Discussion

The rapid accumulation of CO_2 to above 50 per cent, and the corresponding decrease of oxygen to below 4 per cent in 16 hours at 45° C., in a carbon dioxide free atmosphere, and with abundant O_2 supplied, has been shown in fig. 1. It is also quite likely that the percentage of CO_2 in that portion of the tuber which discolors on exposure to the air is much higher, and the O_2 lower than these re-

sults show. BARTHOLOMEW's assumption concerning the accumulation of CO_2 is proved. Whether this is the cause of the death of the cells or not is not yet clear. STEWART and MIX, however, after finding that black heart is produced in a carbon dioxide free atmosphere, conclude that it is the lack of oxygen which is the cause of the death of the cells. BENNETT and BARTHOLOMEW (**8**) also claim support for this idea, stating that the accumulation of CO_2 seems to have no close relation to the production of black heart when potatoes are confined in jars. Unfortunately, however, they did not produce black heart in the absence of carbon dioxide within the tissues affected, nor were the relations of the internal gases found by analysis.

The results of other workers should be cited in this connection. PFEFFER (**19**) states that higher plants are injured by air containing 4–15 per cent CO_2, but believes that partial pressure rather than the percentage of CO_2 is important. CHAPIN (**9**) finds that CO_2 is toxic for plants in general. In their work with the apple, which possesses a tissue similar to that of the potato, MAGNESS and DIEHL (**16**) report that even when sufficient O_2 is present for oxidation, CO_2 accumulation as low as 19 per cent in the intercellular spaces inhibited respiration. Acidity under these conditions decreased. They suggest that since flavors resulting from such conditions are similar to those resulting from absence of oxygen, the action of oxidizing enzymes is inhibited, thus stopping normal oxidation. KIDD and WEST (**14**) have found that CO_2 is a factor in the production of brown heart of apples. Both of the latter workers (**14**, **16**) analyzed the internal atmospheres of the apples used.

One of the most recent pieces of work that has to do with the relation of CO_2 and O_2 in animals is that of SAYERS and YANT (**22**), in which it is shown that a small amount of CO_2 mixed with the O_2 used for the resuscitation of persons affected with CO poisoning caused a quicker recovery than O_2 alone. This was probably due to the stimulative effect of CO_2. The term asphixiation, which BARTHOLOMEW has suggested, and which has been used in the most recent Department of Agriculture Bulletin (**23**) on potato diseases, is not very exact when applied to either plants or animals.

Figs. 2 and 4 illustrate the change in the conductivity. While it is quite possible that conductivity may be a measure of the permeabil-

ity of living tissue, it seems unwise to draw far reaching conclusions from the data at hand. The interpretations of OSTERHOUT (17), who takes the view that conductivity is a measure of permeability, is criticized by STILES and JØRGENSEN (26). They point out that increased conductivity may be brought about by changes which may not affect permeability. For instance, the breakdown of a complex, little ionized substance such as a protein to a simpler, more highly ionized compound such as an amino acid like tyrosine, may increase the conductivity without necessarily affecting the permeability of the tissues involved.

Conductivity, as determined either by the method in which cylinders of tissue were used, or by that in which the electrodes were inserted within the tuber, shows a rise before the fall in resistance. Only suggestions can be made as to the cause of the increased resistance at a period when one would expect it to fall. According to the views of CRILE (10) and OSTERHOUT, it might seem, for example, that the rise of resistance occurring just before death is just what we find as a natural phenomenon,—a sort of false recovery which often occurs shortly before death. OSTERHOUT finds that alkaloids and alcohols among other substances cause a rise before a fall of resistance in *Laminaria* tissues. It may be that alcohol or the alkaloid solanin is released in toxic concentration by the changes which take place in the potato tuber at high temperatures.

One might conclude that since confinement at low temperatures brings about the same breakdown as more rapid respiration at high temperatures, heat itself would not be a factor in producing black heart. Such may not be the case, however, since results of this study indicate that temperature becomes a critical factor in the retention of water by the potato tissues at about 38° C. It is still a question whether the greater amount of water in the inner tissues is sufficient to increase the respiration of those tissues, as the work of BABCOCK (5) suggests.

The curve of catalase activity falls during the period in the oven. Temperature is probably the main factor in this fall. Mrs. RHINE (21) found that catalase activity does not follow respiration in early stages of the germination of seeds of some cereals. LOEW'S theory of catalase action is introduced to explain this difference. Since no lo-

calization of acidity was found, it seems unlikely that catalase activity was affected by acidity changes. RAPER and WORMALL (20), however, have produced evidence which suggests that acidity changes affect the rate of tyrosinase action and the nature of the pigment formed.

It is quite probable that the series of color changes taking place in black heart is the same as those occurring in connection with changes in the potato tuber produced by other means in which death occurs, although the name, black heart, has not been applied to the latter. Among methods producing such color changes may be mentioned killing with the electric current, infection with *Phytophthora erythroseptica* and other disease producing organisms, freezing injuries (JONES 13), and immersion in toluene or glycerine. In fact mere injury, such as crushing, produces the color change. Probably anything which causes the death of the tissues without destruction of the enzyme involved, or alteration of the nature of the substrate, may cause the same changes of color in the tissues killed.

Summary

1. This paper presents the results of a physiological study of black heart of the potato tuber.

2. Observations were made with the purpose of discovering the nature of the physiological changes which must precede or accompany the profound changes that take place in the interior region which suffers the breakdown and undergoes the color changes known as black heart.

3. The disease was produced in the laboratory at a temperature of 45° C., in a carbon dioxide free atmosphere in which abundant oxygen was available. The following determinations were made on the affected tissues over a period of about 16 hours: ratio of carbon dioxide to oxygen in the intercellular spaces, conductivity of the tissues, catalase activity, and H-ion concentration changes.

4. During the time preceding the incidence of black heart, carbon dioxide accumulates rapidly in the internal atmosphere and oxygen is rapidly depleted, until the intercellular gases contain more than 50 per cent of carbon dioxide and less than 4 per cent of oxygen.

5. This change in the internal environment of the cells is the first

change detected, and is followed by increasing permeability of the protoplasm, together with the other changes involved in the development of black heart. At the temperatures used, black heart is apparently the result of high respiratory activity and the failure of the gas exchange to keep pace with respiration rate, but the possible effects of temperature and other factors must not be overlooked.

6. The progress of injury to the protoplasm was followed by electrical conductivity methods, which enable one to judge the degree and rate of injury in any given set of conditions.

7. By these methods it has been shown that there is first a rise of resistance in the tissues, extending over a period which varies with the individual tuber at 45° C. This increased resistance is followed by a continuous fall, beginning with the death of the tissues.

8. These changes are more pronounced with the inner tissues of the potatoes. The cortical regions of the tubers show less change in resistance, and are also much slower to become affected with black heart.

9. The catalase activity of the affected tissues does not seem to be correlated with respiratory activity.

10. During the development of black heart there is a slight increase of H-ion concentration, but this change is not localized. The change as revealed by the indicator method is small and its significance not clear.

11. Temperature may exert a direct effect above 38° C., as some evidence has been obtained that this temperature becomes critical for the maintenance of the normal water relations of the cells.

12. Color changes similar to, or identical with those produced in black heart, may be induced in various ways. Probably anything which causes the death of the tissues without destruction of the enzyme involved, or alteration of the nature of its substrate, may cause the same changes of color in the tissues killed.

Grateful acknowledgment is made to Professor C. A. SHULL for suggesting the problem, and for help and criticism during the progress of the investigation, and also to Dr. S. V. EATON and Dr. G. K. K. LINK for suggestions made in regard to the work.

U.S. DEPARTMENT OF AGRICULTURE
LOS ANGELES, CALIF.

LITERATURE CITED

1. APPLEMAN, C. O., Some observation on catalase. BOT. GAZ. **50**:182–191. 1910.
2. ———, Relation of oxidases and catalase to respiration in plants. Amer. Jour. Bot. **3**:223–233. 1916.
3. ———, Respiration and catalase in sweet corn. Amer. Jour. Bot. **5**:207–209. 1918.
4. ATKINS, W. R. G., Hydrogen-ion concentration of plant cells. Scient. Proc. Roy. Dub. Soc. **16**:414–426. 1922.
5. BABCOCK, S. M., Metabolic water. Univ. Wis. Agric. Exp. Sta. Res. Bull. 22. 1912.
6. BARTHOLOMEW, E. T., Black heart of potatoes. Phytopath. **3**:180–182. 1913.
7. ———, A pathological and physiological study of black heart of potato tubers. Centralbl. Bakt. **43**:609–639. 1916.
8. BENNETT, J. P., and BARTHOLOMEW, E. T., The respiration of potato tubers in relation to the occurrence of black heart. Calif. Agric. Exp. Sta. Tech. Paper no. 14. 1924.
9. CHAPIN, P. Einfluss der Kohlensäure auf das Wachstum. Flora **91**:348–378. 1902.
10. CRILE, G. W., HOSMER, H. R., and ROWLAND, A. F., Electrical conductivity of animal tissues in health and disease. Amer. Jour. Physiol. **60**:59–106. 1922.
11. DEVAUX, H., Étude expérimentale sur l'aération des tissus massifs. Ann. Sci. Nat. Ser. 7. Bot. **14**:297–395. 1891.
12. JOHNSTONE, G. R., Physiological study of two varieties of *Ipomoea batatas*. BOT. GAZ. **80**:145–167. 1925.
13. JONES, L. R., MILLER, M., and BAILEY, E., Frost necrosis of potato tuber. Wis. Agric. Exp. Sta. Res. Bull. 46. 1919.
14. KIDD, F., and WEST, C., Brown heart: A functional disease of apples and pears. Food Investigation Board of Great Britain. Spec. Report no. 12. 1923.
15. MAGNESS, J. R., Composition of gases in intercellular spaces of apples and potatoes. BOT. GAZ. **70**:309–316. 1920.
16. MAGNESS, J. R., and DIEHL, H. C., Physiological studies of apples in storage. Jour. Agric. Res. **27**:1–38. 1924.
17. OSTERHOUT, W. J. V., Injury, recovery and death in relation to conductivity and permeability. Philadelphia and London. 1922.
18. PEARSALL, W. H., and PRIESTLEY, J. H., Meristematic tissues and protein isoelectric points. New Phytol. **22**:185–191. 1923.
19. PFEFFER, W. F. P., Physiology of plants. Oxford. 1897.
20. RAPER, H. S., and WORMALL, A., The tyrosinase-tyrosine reaction. Biochem. Jour. **17**:454–469. 1923.

21. RHINE, LOUISA E., The divergence of catalase and respiration in germination. BOT. GAZ. 78:46–67. 1924.
22. SAYERS, R. R., and YANT, W. P., The elimination of carbon monoxide from blood, by treatment with air, with oxygen, and with a mixture of carbon dioxide and oxygen. Public Health Reprint no. 865. 1923.
23. SHAPOVALOV, M., and LINK, G. K. K., Control of potato tuber diseases. U.S. Dept. Agric. Farm. Bull. 1367. 1924.
24. STEWART, F. C., and MIX, A. J., Black heart and the aeration of potatoes in storage. Geneva, New York Sta. Bull. 436. 1917.
25. STILES, W., and JØRGENSEN, I., Swelling of plant tissues in water and its relation to temperature and various dissolved substances. Ann. Botany 31: 415–434. 1917.
26. ———, Quantitative measurement of permeability. BOT. GAZ. 65:526–533. 1918.

BRIEFER ARTICLES

THE STRIP METHOD FOR SERIAL CELLOIDIN SECTIONS

(WITH ONE FIGURE)

During the past two years, the writer has been engaged in a survey of the vascular anatomy of the buds of a large number of gymnosperms and woody angiosperms. In dealing with such hard and resistant structures as winter buds, it was realized that paraffin would prove an unsatisfactory imbedding medium. Consequently the celloidin method was adopted and satisfactorily met the technical requirements of the problem.

Since the problem under investigation involved a study of the number and course of the bundles constituting the vascular supply of the bud scales and leaves, serial sections were absolutely necessary. Two of the methods recommended for serial celloidin sections were tested. The first method, in which various fixatives were employed in an attempt to fasten the sections on the slides, gave poor results, the majority of the sections dropping off in the staining process. In the second method, which requires the winding of a strip of tissue paper about the sections to hold them in serial arrangement during staining, the paper itself became so deeply colored that any accurate idea of the extent to which the sections were being stained was impossible. The following method was devised after considerable experimentation, and has given uniform results. It appears to be of enough general significance for anatomical work in celloidin to warrant a detailed account.

The material is imbedded in celloidin in the usual manner. When the thickening process has been completed, each bud, surrounded by a considerable quantity of celloidin, is removed from the bottle and molded into a ball-like mass before hardening in chloroform. Preparatory to sectioning, the celloidin ball is trimmed down to a conveniently small cube, an even margin being left on all four sides of the imbedded tissue. This cube is inserted directly in the clamp of the sliding microtome, without previously mounting it on a block of wood.

Each section as it is cut is removed from the knife with a brush and transferred to a clean slide kept moist with 70 per cent alcohol. The sections are placed serially on the slide in rows of five or six each, the size of the block determining the number. The writer has found that 10,

12, 15, or 18 sections are the most convenient numbers to handle on a slide; greater numbers are likely to cause trouble in the staining and mounting processes. When an appropriate number of sections has been cut and transferred in this way, a dissecting needle is used to arrange them on the slide so that their edges just touch without overlapping. The excess alcohol is now removed from the slide by means of a piece of filter paper, which is carefully pressed down upon the sections. Then, with a fine brush, which is kept lubricated in a small bottle of synthol, a 2 per cent solution of celloidin is quickly painted in between the sections. Fig. 1 shows the arrangement of the sections on the slide, the cross-hatching indicating the extent of the celloidin joints. The celloidin should not touch the tissue itself, but must be applied only to the points of contact of the sections. With a little experience, enough celloidin may be carried on the brush at one time to allow the necessary number of horizontal and

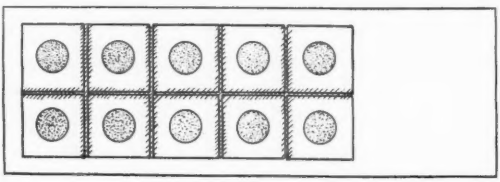


FIG. 1

vertical strokes to be painted, thus insuring smooth, even junctures between the sections. It is important to remove all the alcohol from the slide before applying the celloidin to the sections, as even a slight amount will cause the celloidin to harden on the brush and inevitably produce uneven joints.

The slide is now transferred to a rectangular staining box containing 70 per cent alcohol, to which a small quantity of chloroform has been added. Subsequent groups of sections are similarly treated and placed in the alcohol-chloroform bath until the desired series is complete. Thirty minutes in the bath proved sufficiently long to harden the joints between the sections.

The strips of sections now formed, if not already free from the slides, are removed by carefully inserting the edge of a section lifter between them and the slide while in the bath. A staining box containing 70 per cent alcohol, into which each slide may be separately transferred during this process, is necessary where a great number of strips is being handled. The strips are stored away in boxes of 70 per cent alcohol until

ready for staining. Properly prepared strips were found intact after a month in the alcohol, thus enabling a great number of sections to be prepared in advance of staining.

A number of practical suggestions are now offered in the hope that they may prove of value in other problems involving serial celloidin sections.

1. Thin, flat sections without wrinkles are desirable.

2. Rectangular staining boxes were used because of their adaptability to many phases of the process.

3. The strips should be thoroughly washed in distilled water before treatment with any staining reagents.

4. Chloroform must be added to the absolute alcohol used in destaining and dehydrating to prevent the dissolution of the joints between the sections.

5. In staining, a section lifter is employed in transferring the strips from dish to dish. The excess alcohol should be drained from a strip before transference by carefully touching it to a clean sheet of filter paper.

6. In the problem investigated, Haidenhain's iron-alum haematoxylin and safranin were employed as a double stain. Some difficulty was experienced in removing all the safranin from the celloidin matrix in the destaining process. It was found, however, that a light pink color left in the celloidin does not interfere with the optical properties of the preparation. The effect of other stains on the matrix has not been tested.

7. If thin, smooth junctures have been formed between the sections, the strips will lie fairly flat on the slides for mounting in balsam. Warping apparently is due to variation in thickness of the joints. Lead weights placed on the cover glass of the slides while they are drying are useful in smoothing out wrinkles in the strips.

8. Each strip constitutes a slide. In the anatomical problem investigated by the writer, sufficient changes in size and shape of parts permitted the slides to be arranged in serial order rather easily. In problems where topographical differences are less abrupt and conspicuous in the course of 10–18 sections, a small numbered paper tab may be glued with celloidin to the edge of each strip to indicate the sequence.—ADRIANCE S. FOSTER, *Bussey Institution, Boston, Mass.*

CURRENT LITERATURE

BOOK REVIEWS

Methods of descriptive systematic botany

Recently a new volume by HITCHCOCK has appeared.[1] This work is based upon its author's extensive taxonomic experience during the past 25 years. It contains some 220 pages, divided into 20 portions. Curiously enough, the author appears never to have seen DIELS'S similar work[2] which was published four years earlier. This fact, however, is not without its compensations, for it affords the wholesome assurance that the book reflects the unfettered spontaneity and unswayed judgment of its author.

In the introduction, HITCHCOCK does not confine his efforts to describing the field to be treated. He presents also a discussion of "the scientific attitude." Seldom does one find, in so small a space, so able a treatment of this fundamental feature of all truly scientific work. Various chapters follow, upon categories in the natural system, nomenclature, authors of taxonomic groups, the use of manuals and floras, the identification of plants, etc. Generally speaking, the spirit of treatment throughout the volume is fair and impartial, giving evidence of a judicial temperament and restraint very much to be commended. In the main, the pitfalls of petty controversy have been successfully avoided. As further editions will doubtless be demanded, however, it may be appropriate here to offer certain minor suggestions and comments.

A considerable part of the book is devoted to three "kinds of taxonomic work leading to publication." These are "treated in detail as illustrating progressive steps in technique: the preparation of (1) a local flora; (2) a manual, or descriptive flora; and (3) a revision of a taxonomic group." In the reviewer's opinion, this sequence of the "progressive steps" is open to grave criticism. It is true, of course, that a prospective author should first have gained familiarity with many species in a given flora. But surely, before undertaking to write either a local or a general flora, he should have secured the perspective and judgment that come from intensive and protracted study of one or more small plant groups. In fact, many of the local floras are almost valueless for the very reason that their writer's training in taxonomy had emphasized breadth rather than depth. Again, in the chapter on the preparation of a local flora, we read:

---

[1] HITCHCOCK, A. S., Methods of descriptive systematic botany. 1925.

[2] DIELS, LUDWIG, Die Methoden der Phytographie und der Systematik der Pflanzen (in Abderhalden, Emil, Handbuch der biologischen Arbeits methoden: Abt. XI. Methoden zur Erforschung der Leistungen des Pflanzenorganismus. Teil 1. Heft 2. pp. 67–190). 1921.

"On the basis of critical identification, the local botanist has an opportunity to work out the relationships of species in such a manner that his conclusions will form a valuable contribution to systematic botany." Still again, in the chapter on the preparation of a flora or manual, we read that the author of a flora should form his own judgment as to limits of genera and species. As a purely academic matter, it is probable that most taxonomists, and others too, would prefer to have the setting of these limits left largely in the hands of competent monographers.

The dictum that "the range of a species has no close connection with its abundance" is rather inadequately discussed. On p. 99 occurs the discouraging statement that "the species published since the preparation of the last supplement to the *Index Kewensis* must be found by a patient search through recent literature, especially abstracting journals." The student should be told that at Kew all new specific names are constantly being added to a large card index. In any case of importance, if the genera are known, a written request forwarded to Kew should secure any additional data promptly.

The pleasure derived by the taxonomist in reading the volume inspires the wish that a second edition might be similar in style but more extended in content. This is particularly true in the controversial field of herbaria and herbarium methods. It would have been commendable if HITCHCOCK had devoted more space to the vast importance of herbaria in the study of plant species. Especially does this seem true if one considers the state of neglect into which some of the world's almost priceless herbarium collections have been allowed to fall. At the risk of seeming dogmatic, he might well have taken a more positive stand in the matter of mounting specimens, instead of implying that either strapping or glueing alone was sufficient. Much irreparable damage has been suffered by herbarium specimens just because they had been only glued or only strapped. The chief objective in mounting (that is, the permanency of the specimen rather than, for instance, the beauty of its mounting) ought in the reviewer's opinion to have been emphasized. Furthermore, it is to be regretted that an authoritative set of conclusions by an impartial body of chemists, dealing with the moot but important question of effectively poisoning herbarium specimens, could not have been included. In some herbaria, almost entire families of plants have been ruined by insect ravages. Similarly, many of the common malpractices around a large herbarium appear to have been ignored completely: the use, on labels, of poor ink which fades after a few years; the use, for packets to hold often very valuable fragments, of cheap paper which soon crumbles away; the filing of photographs among the herbarium specimens instead of in separate albums, free from injurious chemicals, etc.

Viewing the book in its entirety, however, the careful reader cannot lay it aside without sensing its great importance as a pioneer work. It will doubtless exert for many years to come a dominant influence for good in its particular field.—E. E. SHERFF.

Mycetozoa

A third edition of LISTER'S *Mycetozoa*[3] will be welcomed by students of this group. The first edition, published in 1894, increased interest in the subject and opened a wide correspondence which brought material from all parts of the world, and made a new edition desirable. This work was begun but LISTER died before it could be completed. His daughter, however, who had been constantly associated with him during the preparation of the first edition and the revision for the second, and who had painted many of the plates which are such an important feature of the work, continued the revision and made possible the second edition which appeared in 1911. In the third edition, three new genera and 46 new species are described, and 22 new plates have been added, 8 of which are colored. The keys are very complete and there is an extensive bibliography.

It is due to Miss LISTER'S generosity that a book with 223 plates, 126 of which are colored, can be sold at a price within the reach of those who need the monograph.—C. J. CHAMBERLAIN.

NOTES FOR STUDENTS

Fluorescence of chlorophyll.—Our knowledge of the mechanism of photosynthesis increases slowly but steadily. In recent years it has been shown to be an adsorption process, and various workers have endeavored to solve some of its mysteries by the use of artificial systems with ultra-violet light. The chlorophyll itself, however, has received scant attention since WILLSTÄTTER'S masterly researches made known its composition. NOACK[4] has attacked the problem from the pigment side, and has attempted to demonstrate that the fluorescence of the chlorophyll is definitely correlated with its photosynthetic activity. The thesis is not a novel one, for TSWETT advanced it fifteen years ago; but it is ably presented, with a quantity of interesting experimentation detailed in its behalf, and plentiful reference to the work of others which seems to support it. Unfortunately few but German citations are made. The questions are raised, whether illumination of fluorescing chlorophyll raises the oxidation potential of the system, and whether the energy change is of an order comparable with that of the work done in photosynthesis. Both these questions the author believes he can answer affirmatively.

The photo-oxidation catalyzed by fluorescing pigments was demonstrated qualitatively with benzidine, and quantitatively by the measurement of the consumption of O_2. Benzidine is readily oxidized on illumination in the presence of fluorescing dyes, such as eosin, in both aqueous and organic solvents, and as its brown oxidation product is readily detected, it proved very suitable for the

---

[3] LISTER, ARTHUR, revised by LISTER, GULIELMA, A monograph of the mycetozoa, a descriptive catalogue of the species in the herbarium of the British Museum. 800 pp. xxxii+296. *pls.* 223. 56 wood cuts. British Museum, London. 1925. £1. 11s. 6.

[4] NOACK, K., Photochemische Wirkungen des Chlorophylls und ihre Bedeutung für die Kohlensäureassimilation. Zeitsch. Bot. **17**:481–548. 1925.

work in hand. Chlorophyll in true solution behaved like other fluorescing dyes, but its colloidal dispersion and solutions of Cu-chlorophyll, neither of which fluoresce, had no photo-oxidative effect. Furthermore, chlorophyll in both living and dead leaves caused photo-oxidation, and STERN has reported it as fluorescent in leaves, though WILLSTÄTTER had thought it colloidal.

Further evidence is found in a study of the bleaching of chlorophyll, a process which has often been shown to be an oxidation. The author finds that this bleaching is greatly delayed in the presence of benzidine, Na_2SO_3, the carotinoids, and other good acceptors of O^2, and offers the explanation of bleaching as an auto-photo-oxidation of the chlorophyll in the absence of better O_2-acceptors. Again the colloidal chlorophyll and the Cu-derivative are relatively photostable.

It is suggested that in photosynthesis CO_2 is the normal acceptor of the energy absorbed by chlorophyll on illumination. When the cell is deprived of CO_2, either directly or by traces of phenylurethan, which is preferentially adsorbed and so has the effect of excluding it, there may occur not only bleaching of the chlorophyll, but also an injury of the protoplasm by the well known photodynamic effect, first shown with bacteria by HERTEL. SO_2 has long been known to exercise a special toxicity for green cells, and it is found to have an effect similar to that of phenylurethan, so that an explanation of this toxic action is afforded on a photo-dynamic basis. In all these cases the chlorophyll absorbs radiant energy and must get rid of it again. In the absence of the normal acceptor, or of easily oxidized substitutes, the pigment and the protoplasm must be oxidized under the influence of the oxidation potential which has been built up.

The least satisfactory part of the paper is the theoretical portion, dealing with energy transformations. The author believes that WARBURG'S "primary photo-chemical product" is a peroxide, and has obtained in an artificial system the same results as WARBURG with green cells. In the absence of CO_2, the photo-energized chlorophyll molecule is believed to react with O_2, forming this peroxide, which then oxidizes the chlorophyll. The energy of this reaction is supposed to induce a chemi-luminescent fluorescence on the part of a second chlorophyll molecule. In the presence of CO_2, however, the energized chlorophyll is supposed to react with it, effecting its reduction, and again the energy of the reaction is called upon to cause the fluorescence of a second molecule of the chlorophyll. Here the primary peroxide formation is gracefully forgotten. As an alternative it is suggested, on the basis of some inorganic experiments of WIELAND, that the first step may be the activation of H_2 by transfer to it of energy from the chlorophyll. This activated H_2 may either react with CO_2, reducing it, or in absence of CO_2, with O_2 to form H_2O_2, which oxidizes the chlorophyll. Quite apart from the very involved mechanism of fluorescence, as here conceived, the theories encounter serious difficulties when the questions of actual electron and energy transfer are considered. The author does not enter into any details, but rather patches together theories with pieces extracted from the work of various investigators in other fields.

There are many instances in the work where the conclusions drawn are

based on insufficient evidence. The proof offered of a linear relation between O_2-consumption and O_2-partial pressure is open to question, because too few points were determined on the curve shown to assure that it really does not pass through the origin, as a straight line would not. Also, the demonstration of the equality of the energy change in illuminated chlorophyll and in photosynthetic work is possible only by considerable juggling of the few data obtained. Again, there is much stress laid on the fact that Cu-chlorophyll, which does not fluoresce, does not cause photo-oxidation. This observation would be much more valuable if it had been shown that the similarly derived Zn-chlorophyll, which does fluoresce strongly, was photo-oxidative, for the treatment producing these substituted phaeophytins may have produced intra-molecular changes.

In spite of these and other criticisms, however, the author has certainly shown that there is a definite relation between the fluorescence of chlorophyll and its photo-oxidative action, and he has given food for thought as to whether this relation may not be in some way of primary importance in photosynthesis, since the photo-oxidation is only the manifestation of an increased energy potential which can be diverted, perhaps, to photosynthetic use by CO_2. A new mode of attack has been suggested, and some problems already solved thereby. —H. S. WOLFE.

Island floras.—The great amount of endemism of many of the Pacific islands is a matter that has been known to botanists for years. The explanation of this phenomenon, and more particularly the accounting for the relationships existing between the floras of the different groups, presents many difficulties. Thus Juan Fernandez and Hawaii are volcanic islands remote from each other, but with rather closely related floras. SKOTTSBERG has studied the former rather critically,[5] and has now made a careful comparison between the two.[6] He concludes that it is improbable that the vegetation has originated on the islands since they assumed their present shape, for they are young islands and the floras show evidence of considerable age. He presents evidence that seems to indicate that the Juan Fernandez flora, at least, is of continental origin, and that it existed long before the islands were formed. The flora seems to have gradually taken possession of the islands as the continent became submerged. A part of the flora has come from South America, thus explaining affinities with New Zealand and Polynesia. Hawaii also contains Antarctic types whose original home must have lain far to the south, and it seems probable that the history of the Hawaiian flora was similar to that of Juan Fernandez.—GEO. D. FULLER.

---

[5] SKOTTSBERG, C., Natural history of Juan Fernandez and Easter Island. **2**: pp. 238 Upsala. 1922.

[6] ———, Juan Fernandez and Hawaii: A phytogeographical discussion. Bernice P. Bishop Museum Bull. **16**:1–47. 1925.

Studies on Nematospora.—WINGARD.[7] has published a valuable account of his investigation of *Nematospora Phaseoli*, one of the yeasts. It belongs to the group with needle-shaped ascospores, and was described originally as budding in the cotyledons of hazelnuts, producing asci from individual bud cells. The conclusions reached by WINGARD are briefly as follows. He finds that the genus is parasitic on a wide range of plants, but especially on the Leguminosae, and that infection is associated with the punctures of particular insects. Although the vegetative phase is usually yeastlike, in certain media it produces a rudimentary mycelium. In ascus formation it conforms to the usual Ascomycete program as to divisions and number of ascospores. The cytological details of nuclear division are also presented. As to relationships, the conclusion is reached that it is closely related to *Monospora* and *Coccidiascus*, so that these three genera form a natural group of Saccharomycetes.—J. M. C.

The flower of Euphorbia.—Miss HABER.[8] has attacked the difficult problem of the affinities of the Euphorbiaceae, which family "modern botanists place in the Geraniales." The previous conclusions have been drawn mostly from morphology and ontogeny, which Miss HABER has checked up with a study of the anatomy of the floral structures. The flowers of 33 species were studied, the specimens representing widely separated habitats. After presentation of the evidence, the conclusion is reached that the so-called "flower" represents an inflorescence in an advanced stage of reduction. The "involucre" comprises a whorl of alternate bracts and pairs of secondary branches of the inflorescence. The "ovary" is a pistillate flower terminating the main axis of the inflorescence. The process has been one of aggregation, suppression, and cohesion in the inflorescence. This certainly shows that the genus is very highly specialized, with extreme complexity and congestion and consequent reduction.—J. M. C.

Chemical bibliographies.—Valuable assistance to research is being rendered by the National Research Council through its publication of important bulletins. Attention is called to a recent bulletin compiled by WEST and BEROLZHEIMER,[9] for the Research Information Service of the Council. It consists of a bibliography of bibliographies on chemistry and chemical technology, covering the period from 1900 down to the close of 1924. It does for chemistry what previous bulletins have done for geology and physics. The first two sections list

---

[7] WINGARD, S. A., Studies on the pathogenicity, morphology, and cytology of *Nematospora Phaseoli*. Bull. Torr. Bot. Club **52**:249–290. 1925.

[8] HABER, JULIA M., The anatomy and the morphology of the flower of *Euphorbia*. Ann. Bot. **39**:657–607. 1925.

[9] WEST, C. J., and BEROLZHEIMER, D.D., Bibliography of bibliographies on chemistry and chemical technology, 1900–1924. Nat. Res. Council Bull. 50. 8 vo. pp. 308. Washington. 1925.

the general bibliographies, and the abstract journals and year books. Part III lists the general indexes of serials, but the important section for chemists and biologists is section IV, which makes up almost the entire volume. It is an alphabetically arranged bibliography of special subjects. For instance, one finds almost a hundred bibliographies on metabolism, ten on hydrogen-ion concentration, about seventy on colloids, eighteen on photosynthesis, ten on respiration, etc. Of course the biologist will not use many of the lists, but these examples show how useful such a compendium may be to the investigator. There is a small group of personal bibliographies at the end of the volume.

The subject headings number about 2400, with about 10,000 bibliographies from 7500 authors. Naturally one could not expect a work of this kind to avoid omissions, but it will furnish a convenient starting point for investigation of the literature of the first twenty-five years of the century, so far as chemistry and chemical technology are concerned. The reviewer considers it a valuable addition to our source books of research.—C. A. SHULL.

Electrostatic theory of permeability.—About three years ago the writer[10] proposed an electrostatic theory of permeability based upon various results obtained in permeability studies previously published. This theory stated briefly that the effect of a salt depended: (1) upon the electrostatic charge on the colloidal elements or particles which make up the plasma membrane; and (2) upon the charges on ions of the penetrating salt, which must be considered individually. A cation with two positive charges is much more effective than two anions with single charges each. Antagonism was proposed as existing between cations and anions, and it was shown that, assuming that the protoplasm is negatively charged, the effect of a salt can be largely foretold in its effect upon the permeability of the protoplasm. In addition, most of the antagonistic effects reported up to that time were explained upon the basis of this theory.

In the last three years much additional work has been done by various investigators, and it is the purpose of this review to report the progress of the theory and to correlate with it as much as possible of recent important work, as well as to acknowledge contributions not previously mentioned.

In connection with the supposition that the protoplasm is negative in charge, it may be said that this rested upon the fact that the sea water and the blood plasma are both slightly alkaline, which would lead one to expect negative charges on the layers immediately in contact with these fluids. Further, the cataphoresis of blood corpuscles toward the positive pole indicated the presence of a negative charge.

Of the work done since the writer's paper appeared, of prime importance is that of HEILBRUNN,[11] who has brought evidence to show that the interior of the

---

[10] RABER, O., Permeability of the cell to electrolytes. BOT. GAZ. **75**:298–308. 1923.

[11] HEILBRUNN, L. V., The colloid chemistry of protoplasm. I. General considerations. II. The electrical charges of protoplasm. Amer. Jour. Phys. **64**:481–498. 1923.

———, The electrical charge of living cells. Science **61**:236–237. 1925.

cell is charged positively and the exterior negatively. This has been concluded from the measurement of changes in the fluidity of the internal protoplasm. Ca and Mg ions make the inner surface more fluid than monovalent ions, while Ce and Al are still more active. Thus cations have the same loosening effect upon the inner protoplasma which anions have upon the outer (as found in *Laminaria*). HEILBRUNN supports the electrostatic theory completely, and agrees that the antagonistic action of salts is to be explained by their effects upon the viscosity of protoplasm. He explains the difference in sign of the outer and inner protoplasm as due to the rate of diffusion from the surface of HCO_3 and CO_3 ions, which he thinks would account for the difference in electrical charges. This simply means that the interior of the cell is acid because it is in contact with the products given off by catabolism which result in acid solutions. The exterior is alkaline because it is in contact with an alkaline solution. Most determinations of the P_H of the cell sap show it to be acid, which supports this hypothesis.

Another interesting support comes from the work of BROOKS,[12] who finds that pentavalent arsenic penetrates the protoplasm and goes through into the cell sap of *Valonia* less easily than the trivalent form. This is comparable with the writer's work on iron in its bivalent and trivalent forms.

More recent work has been reported by SCHAEDE,[13] who finds by the use of stains that the living protoplasm in the onion is basic, while dead protoplasm and cell sap have an acid reaction. He suggests that the dead protoplasm may not be acid in itself, but is made so by the acid cell sap.

In the work on antagonism and permeability many papers support the electrostatic hypothesis. As examples of these only a few of the more important will be cited.

An interesting paper in this connection is that of FREE,[14] which, although published before, did not come to my attention until after my theory had been published. He suggests that the state of aggregation of the particles of the protoplasmic membrane is the determining factor in permeability. He does not suggest that electrostatic effects are of prime importance in bringing about these changes, but the paper is mentioned here so that the literature may be complete.

A more suggestive paper by GREEN[15] appeared about the same time. He worked with gelatin, and suggested from the results obtained that balanced solutions were important in plant growth because they were in equilibrium with iso-electric protein. We see now that they are in equilibrium, not with iso-

---

[12] BROOKS, M. M., The penetration of trivalent and pentavalent arsenic into living and dead cells. Proc. Soc. Exp. Biol. and Med. **21**:50–51. 1923.

[13] SCHAEDE, R., Über die reaktion des lebendes Plasmas. Ber. Deutsch. Bot. Gesells. **42**:219–223. 1924.

[14] FREE, E. E., A colloidal hypothesis of protoplasmic permeability. Plant World **21**:141–150. 1918.

[15] GREEN, N. B., The effect of ions of NaCl and $CaCl_2$ upon the electrical conductivity of certain colloidal mixtures. Plant World **21**:303–316. 1918.

electric protein, but with protoplasm which is negative in charge; but GREEN was correct in connecting permeability with electrostatic charges. This paper of GREEN's is connected with previous work of FENN,[16] to whom GREEN expresses his indebtedness. FENN had studied the precipitation of gelatin by alcohol and the antagonistic effect of salts upon this process. He saw the importance of electric charges in this connection, and accepted the electrostatic explanation of oppositely charged ions as the simplest one for the antagonistic effects observed.

The writer's hypothesis has explained the ordinary antagonism between NaCl and $CaCl_2$, but examples are constantly recurring in the literature, such as that reported by WINSLOW and FALK[17] for *Bacterium coli*. In dilute solutions of five parts NaCl and one of $CaCl_2$, not only is all toxicity destroyed but there is even a stimulating effect observed.

CHOLODNY[18] finds that electrolytes increase the viscosity of protoplasm, which he attributes to the action of the cation on the negatively charged disperse phase. Polyvalent anions inhibited this cation action.

SCARTH[19] finds that the permeability effects in *Spirogyra* depend upon the valency of the ion. The method he used for determining penetration was one which measured *ultimate* penetration, and does not separate it from the first effect, which may be a decrease of permeability. The paper is very suggestive, however, and can easily be interpreted as a support for the electrostatic hypothesis.

By far the most important work in this field has been done by KAHO,[20] who was publishing at the same time as the writer. KAHO's summary (condensed) is as follows:

"1. The penetration of neutral salts into plant protoplasm is a physicochemical process, which depends upon the colloidal activity of the salts in which both ions are important.

"2. The penetration of the salt depends upon its ability to change the colloidal conditions of the plasma membrane, in which the action of each salt is

---

[16] FENN, W. O., Similarity in the behavior of protoplasm and gelatin. Proc. Nat. Acad. Sci. **2**:539–541. 1916.

[17] WINSLOW, C. E. A., and FALK, I. S., Studies on salt action. IX. The additive and the antagonistic effects of sodium and calcium chlorides upon the viability of *Bact. coli*. Jour. Bact. **8**:237–244. 1923.

[18] CHOLODNY, N., Über Protoplasmaveränderungen bei Plasmolyse. Biochem. Zeitsch. **147**:22–29. 1924.

[19] SCARTH, G. W., The penetration of cations into living protoplasm. Amer. Jour. Bot. **12**:133–148. 1925.

[20] KAHO, H., Ein Betrag zur Permeabilität des Pflanzenplasmas für die Neutralsalze IV. Biochem. Zeitsch. **123**:284–303. 1921.

———, Über die physiologische Wirkung der Neutralsalze auf das Pflanzenplasma. Bull. no. 18, Bot. Inst. Dorpat Univ. 1–167. 1923.

the additive effect of the oppositely acting ions. The kations have a coagulating effect which lowers the permeability for the salt. The anions have a peptising or dissolving action on the plasma colloids, which increases the permeability of the salt. The resulting action of a neutral salt is the algebraic sum of these opposite tendencies.

"3. The toxicity of a salt is the result of its penetrability.

"4. The lipoid materials of the surface layers of the plasma play an important part in the intake of neutral salts, thus supporting the work of HANSTEEN-CRANNER[21]."

KAHO's work and my own have both stressed the fact that antagonism is between cations and anions rather than between cations alone, as had been commonly held formerly, but KAHO does not emphasize electrostatic effects quite so much or give a definite picture as to how these electrostatic differences are effective.

KAKIUCHI,[22] in work done on the precipitation of phospholipin, obtains results similar to KAHO and the writer. He agrees that the antagonism is between cations and anions, but holds that the lipoids of the plasma membrane are the responsible colloids, in which he may be correct. The electrostatic hypothesis as formulated by the writer did not deal with this aspect of the matter. In case the lipins are the materials of the membrane which are affected by the salts, many of the phenomena observed by OVERTON could be correlated still further than when the electrostatic hypothesis was proposed. The findings of KAKIUCHI and HANSTEEN-CRANNER, in fact, link up the electrostatic theory with the lipoid theory in a very interesting and suggestive manner.

MANN[23] finds that basic dyes are absorbed more than acid by mangold tissue, that is, that the tissue is more permeable to basic dye, as might be predicted. The dye intake is antagonized by salts and the antagonism is a function of the valency of the cation, just as the intake of acid or positive dyes is antagonized by anions, and varies with their valency. This work thus supports the colloid precipitation theory, and the electrostatic theory of permeability.

MACDOUGAL,[24] [25] who has worked extensively on the penetration of salts into succulent tissues and their effects upon permeability, has taken a decided stand for a colloidal, electrostatic explanation of permeability phenomena, and

---

[21] HANSTEEN-CRANNER, B., Beitrage zur Biochemie und Physiologie der Zellwand und der plasmatischen Grenzschichten. Ber. Deutsch. Bot. Gesells. **37**:380–391. 1919.

[22] KAKIUCHI, S., Studies on physico-chemical properties of phospholipin. Jour. Biochem. Tokyo **1**:165–174. 1922.

[23] MANN, C. E. T., The antagonism between dyes and inorganic salts in their absorption by storage tissue. Ann. Botany **38**:753–777. 1924.

[24] MACDOUGAL, D. T., Permeability and the increase of volume of contents of living and artificial cells. Proc. Amer. Phil. Soc. **62**:1–25. 1923.

[25] ———, Growth and permeability. Carn. Inst. Year Book **23**:125–131. 1924.

says: "The most useful explanation which has as yet been offered in this matter is that of RABER, in which these effects are taken to rest upon the relative density of the charges upon the kations and anions acting upon the colloids."

Many other articles from recent literature could be cited, but these are sufficient to show the kind of support now given to the electrostatic theory from various angles, and to show that this hypothesis gives promise of extreme utility, whether the colloid particles concerned are lipoid, protein, or both.—O. RABER.

Taxonomic notes.—REHDER[26] has published some results of his investigation of the material assembled at the Arnold Arboretum. The result is a description of 1 new species, 8 new varieties, 17 new combinations, 17 new forms, and 4 new names of shrubs and trees.

WILSON[27] has published a detailed account of the Taxaceae and Pinaceae of the Yunnan province of China, from material collected by J. F. ROCK under the auspices of the National Geographic Society. The Taxaceae are represented by 8 species, belonging to *Taxus*, *Podocarpus* (3 spp.), *Torreya*, and *Cephalotaxus* (3 spp.). The Pinaceae include 33 species in 15 genera, Yunnan being richer in this group than any other region of China, and also including 3 interesting endemic species.

WELCH[28] has published a monograph of the North American representatives of *Cucurbitaria*. He recognizes 25 species, and excludes 43 species previously listed under the genus.

BURT,[29] in continuation of his investigation of the Thelephoraceae of North America, has published a very detailed account of the genus *Peniopora*. Full record is made of distribution and of material examined. He recognizes 120 species, 67 of which are described as new. There are also 11 new combinations.—J. M. C.

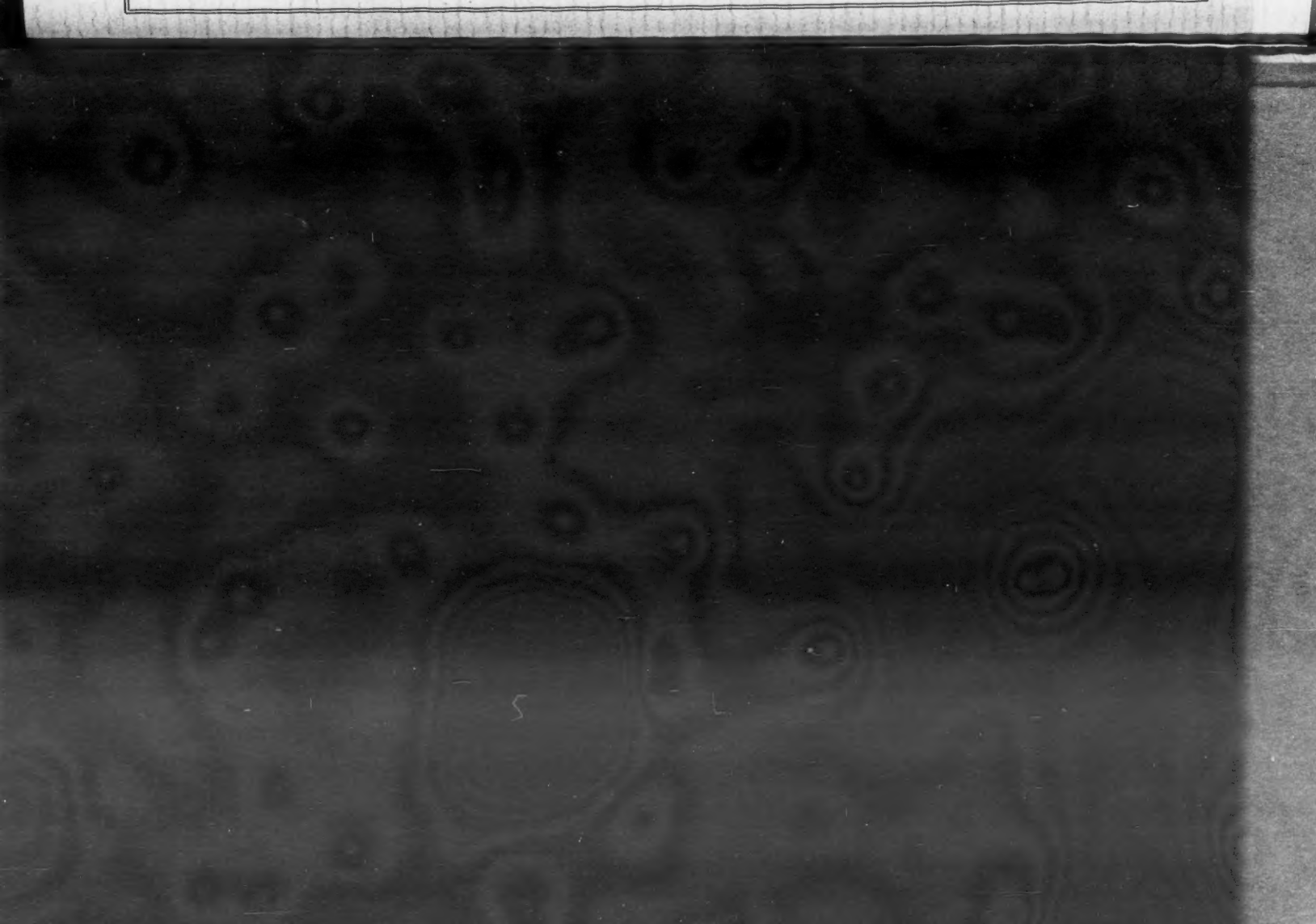
---

[26] REHDER, ALFRED, New species, varieties, and combinations from the herbarium and the collections of the Arnold Arboretum. Jour. Arnold Arboretum **7**:22–37. 1926.

[27] WILSON, E. H., The taxads and conifers of Yunnan. Jour. Arnold Arboretum **7**:37–68. 1926.

[28] WELCH, D. S., A monographic study of the genus *Cucurbitaria* in North America. Mycologia **18**:51–86. 1926.

[29] BURT, E. A., The Thelephoraceae of North America. XIV. Ann. Mo. Bot. Gard. **12**:213–357. 1925.



Cambridge University Press

Monocotyledons. A Morphological Study. By AGNES ARBER, M.A., D.Sc. With a frontispiece and 160 illustrations. Large Crown 4to. $7.00. (*Cambridge Botanical Handbooks.*)

Devonian Floras. A Study of the Origin of Cormophyta. By E. A. NEWELL ARBER, M.A., Sc.D. With a Preface by D. H. SCOTT, F.R.S., a frontispiece, and 47 text-figures. Demy 8vo. $6.00.

The Classification of Flowering Plants. By ALFRED BARTON RENDLE, M.A., D.Sc., F.L.S., Keeper of the Department of Botany, British Museum. Vol. II. Dicotyledons. With 279 illustrations. Demy 8vo. $8.00.

A Dictionary of the Flowering Plants and Ferns. By J. C. WILLIS, M.A., Sc.D., F.R.S. Fifth edition, revised. Crown 8vo. $9.50. (*Cambridge Biological Series.*)

British Grasses and Their Employment in Agriculture. By S. F. ARMSTRONG, B.A., F.L.S., of the School of Agriculture, Cambridge. With 175 illustrations. Demy 8vo. $4.25.

The Ferns (Filicales). Treated comparatively with a view to their natural classification. By F. O. BOWER, Sc.D., LL.D., F.R.S. Vol. I, Analytical Examination of the Criteria of Comparison. With a frontispiece and 309 illustrations. Royal 8vo. $10.00.

Plant Life on East Anglian Heaths. Being Observational and Experimental Studies of the Vegetation of Breckland. By E. PICKWORTH FARROW, M.A., D.Sc. With 46 illustrations. Crown 4to. $2.50.

The Anthocyanin Pigments of Plants. By MURIEL WHELDALE ONSLOW, M.A. Second edition. Royal 8vo. $7.00.

Experiments in Genetics. By CHARLES C. HURST, Ph.D. (Cantab.). With 174 illustrations and a map. Large Royal 8vo. $16.50.

Botany: A Junior Book for Schools. By R. H. YAPP, M.A. (Cantab.), Mason Professor of Botany in the University of Birmingham. Second edition. With 159 text-figures. Crown 8vo. $1.20.

Botany: A Textbook for Senior Students. By D. THODAY, M.A., Lecturer in Physiological Botany, University of Manchester. Third edition. With 230 text-figures. Crown 8vo. $3.50.

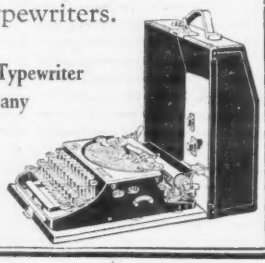
Life. An Outline of Biology. By SIR A. E. SHIPLEY, G.B.E., F.R.S. Second edition. With 71 illustrations. Crown 8vo. $2.50.

Published by the Cambridge University Press (England)
The Macmillan Company, Agents in the United States
60 Fifth Avenue, New York City

Remington Portable

THE handy little friend that makes all writing swift and easy.

The smallest, lightest, and most compact of all standard keyboard portable typewriters.



Remington Typewriter Company

374 Broadway
New York

Branches Everywhere

OUTLINE OF GENETICS

With Special Reference to Plant Material

By MERLE C. COULTER

The basis for this study is found in *Plant Genetics*, which the author of the present volume published a few years ago with his father, Dr. John M. Coulter. Complete revision and reorganization of the original material enables this work to continue to meet the needs of botany students, both beginning and advanced.

$1.50, postpaid $1.60

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - ILLINOIS

GENERAL CYTOLOGY

A Textbook of Cellular Structure and Function
For Students of Biology and Medicine

***Edited by* E. V. COWDRY**

The first co-operative and comprehensive attempt by specialists in research fields to state in general terms what is known or conjectured regarding the principles which govern cellular structure and function. It is the first volume to present the data concerning the cell that are fundamental not only to the sciences of botany and zoölogy, but also to physiology and pathology.

Thirteen eminent scientists have contributed to its preparation: Robert Chambers, E. G. Conklin, E. V. Cowdry, M. H. Jacobs, E. E. Just, Margaret R. Lewis, W. H. Lewis, Frank R. Lillie, Ralph S. Lillie, C. E. McClung, A. P. Mathews, T. H. Morgan, and E. B. Wilson. Each has written on that part of the subject which has occupied his own research, and the result is a more comprehensive and authoritative presentation of cytology than has ever before been attained.

Royal 8vo, 754 pages, illustrated, $7.50, postpaid $7.75

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO · ILLINOIS